Nutritional Ecology of Brushtail Possums (*Trichosurus vulpecula* Kerr) in New Zealand

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Abstract

In this thesis, a combination of field observations and laboratory experiments are used to address the question: how do the nutritional strategies of brushtail possums enable this alien pest species to obtain a balance of nutrients at native forest-pasture margins in New Zealand?

Possums had a polyphagous diet with a core of leaves from three species, supplemented by flowers in spring and summer, fruits in autumn and invertebrates in winter. Graphical models confirmed that possums were polyphagous in all seasons, although diet width and the extent to which individual species of food dominated the diet varied between seasons.

Four nutrient characteristics of potential foods were analysed: total nitrogen, available nitrogen, soluble organic matter and total digestible dry matter. They varied significantly between food species and between seasons but were not significantly associated with either proportion by mass, or frequency of occurrence in the diet.

In laboratory experiments relative preferences between leaves of three woody species changed when the basal leaf diet was supplemented by flowers or by pasture species. Intake rates of nutrients also varied according to diet. The target nutrient intake ratio of available nitrogen to digestible dry matter was calculated. Predictions from optimal foraging, nitrogen limitation and mixed diet theories were compared but mixed diet theory was supported most strongly.

Home ranges were delineated using data from GPS telemetry. Nocturnal foraging pathways were characterised and divided to distinguish between searching within patches and travelling between them. Possums travelled further per night and travelled faster between search patches in winter than they did in autumn. Selection ratios showed that native forest and native shrub/scrubland were preferred habitats for both travelling and searching.

The implications of applying mixed diet theory to possum nutritional ecology, for improving the efficiency of control programmes and reducing interactions between possums and cattle are explored.

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Chapter 1: Introduction

1.1 Philosophical background

A dichotomy between holistic and reductionist approaches to thinking is evident in a considerable diversity of human endeavours. Ecological science exists along this reductionist-holistic continuum with nested levels of study ranging from cells, individual organisms, populations, communities, ecosystems to the biosphere as a whole (Wiegert 1988). Ecology is therefore complex and has a particularly diverse remit. Because of this, there is a wide variety of sub-disciplines within ecology that have a variety of goals and methodological approaches. This has influenced the choice of how data should be collected and questions should be answered: e.g. by field observations, field experiments, laboratory observations or laboratory experiments. Studies in which field observations are used to scrutinise phenomena in a wider, complex environment full of interacting parts, are often holistic. Studies in which laboratory experiments are conducted are at the opposite end of this continuum because in these, small parts of a biologically complex whole are examined in isolation from the whole natural environment in which they normally exist.

Overall, no one approach can be seen as superior because all come with simultaneous advantages and disadvantages. At the reductionist end, the ability to extrapolate to the field is compromised for the sake of increasing levels of control and precision. Despite increased realism, in holistic field studies confounding variables can be a major challenge when studying whole complex systems. Therefore, answering ecological questions often requires employing a variety of methodological approaches, and philosophical constructs, in complimentary ways.

1.2 Nutritional ecology

Fundamental ecological questions about how organisms acquire the resources that are vital to their survival, growth and reproduction have long fascinated ecologists (Elton 1927). Nutritional resources influence the distribution and abundance of organisms, as well as influencing how animals interact with each other and their environment in order to acquire the resources needed to sustain

life (Raubenheimer et al. 2009). Therefore, '*how do organisms acquire food and absorb nutrients'* is a fundamentally important question in ecology.

1.3 Foraging strategies

Foods available to heterotrophs contain mixtures of some or all of the major classes of essential nutrients, in differing proportions, together with toxins and digestibility reducing compounds (Foley & Moore 2005). Foraging strategies comprise a suite of tactics relating to locating and acquiring a balanced nutrient uptake from these foods (Stephens & Krebs 1987). Animals face choices about where to feed, what prey items to consume, how long to spend feeding in different patches of prey or on different food items. How animals have evolved to solve these problems, have been at the core of behavioural ecology since its inception (Tinbergen 1951; Tinbergen 1963). A fundamental assumption, derived from evolutionary theory, is that selection acts to favour individuals with a combination of foraging and digestive strategies that maximise phenotypic fitness (Krebs & Davies 2009).

The focus of this thesis is on vertebrate herbivores. Vertebrate herbivore foraging behaviour is not simply influenced by the quality or nutritional contents of potential foods (DeGabriel et al. 2014). Other influencing factors include; the availability of foods within the landscape (e.g. Vivas & Saether 1987), the spatial and temporal scale of the heterogeneity in plants and other potential foods (Wiggins et al. 2006), the anti-feedant properties of potential foods (Dearing et al. 2005), competition with conspecifics (e.g. Symington 1988), and the availability of non-food resources e.g. shelter (Godvik et al. 2009). In addition to plant-herbivore interactions, predator-prey interactions also influence herbivore foraging decisions (Kirmani et al. 2010). For example, predation risk had a significant effect on brushtail possum foraging behaviour in Australia (including feeding time, feeding rate, intake rate and vigilance) (Nersesian et al. 2011). However, the effect of plant toxins in food (*Eucalyptus* spp.) was greater than the effect of fear (from both direct and indirect cues) in reducing patch residence time (Kirmani et al. 2010).

Because the majority of vertebrate herbivores cannot obtain their entire nutrient requirements from a single species of food plant, multiple species diets/ polyphagy, is extremely common (Simpson & Raubenheimer 2012). Therefore, a

critical trade-off for herbivores is in locating multiple patches of different resources in environments that are heterogeneous in terms of the availability and nutrient status of foods they contain. Herbivores forage in patchy environments (Kotliar & Wiens 1990) with several 'nutritional dimensions' i.e. fitness influencing nutrients (Simpson & Raubenheimer 1993).

Patchiness occurs at a range of spatial scales, from differences in nutrient concentration between different tissues within individual foods, to differences between areas with different compositions of potential food species. There are also temporal changes in the environment; a seasonal change in the quality or relative availability of particular food alters the relative quality of different food patches (Senft et al. 1987).

A range of foraging strategies enables herbivores to overcome the dietary constraints placed upon them by the variation in availability and quality of their food source (Wiggins et al. 2006). Foraging behaviour is also expressed over several spatial and temporal scales from bites and steps within a patch, to movements that connect food patches and movements that generate home ranges (Senft at al. 1987; Owen-Smith et al. 2010). For example, the spatial and morphological characteristics of plants influence the intake rate of plant tissues within patches (Spalinger & Hobbs 1992). The size and density of foraging patches also influence herbivore foraging strategies; time spent foraging within a patch has been shown to increase as patch size increased and as distance between patches increased (Shipley & Spalinger 1995). For herbivores, food intake rate has been shown to vary between plant species and growth forms (Trudell & White 1981). Similarly, bite rate and bite size also vary between different plant types and are dependent on plant biomass (Trudell & White 1981). The size of individual plants and their dispersion in the landscape impact on searching and handling time (Laca et al. 1994) which are key components of foraging behaviour. Furthermore, patch size and density also influence intake rate (Shipley & Spalinger 1995). However, there is an upper limit on intake rate which is constrained not by intake rate, but by the animal's capacity to process the food in the short term (Gross et al. 1993).

Therefore herbivores experience and respond to differences in the availability and profitability of foods at different spatial and temporal scales. Because generalist herbivore must switch between different food types to maximise their intake then the degree of plant heterogeneity and the spatial scale at which it occurs is likely to influence herbivore foraging behaviour (Wiggins et al. 2006).

1.4 Plant defence

Plants have evolved a range of strategies to defend themselves from herbivory, on both a physiological and a chemical basis (Dearing et al. 2005). Plant defences can either be constitutive (always present in the plant) or induced at the site of injury or attack. Constitutive defences vary widely and range from low nutrient status (Moran & Hamilton 1980) and mechanical defences (Massey et al. 2007) to digestibility-reducing or toxic compounds (Foley & Moore 2005). Herbivores possess a range of specialisations that enhance the breakdown of fibrous cell walls. In particular, through mechanical digestion (chewing), chemical digestion and biological digestion by gut microbes. All herbivores have specialised dentition and the digestive system itself is large and well developed. The large size, volume and surface area of the alimentary track increases the retention time of foods; this increases the time for chemical and biological digestion and increases absorption over a larger surface area (Hume 1999). A further digestive strategy used by herbivores to meet their requirements from a highly fibrous diet is to maximise their intake, subject to body size. Because energy requirements per kilogram of body mass increase with decreasing body size (Montague and Van Soest 1985), this theoretically sets a lower limit to the size of purely herbivorous mammals (Clauss et al. 2003).

Induced defences in plants include production of secondary metabolites as well as morphological and physiological changes (Tuomi et al. 1984; Chinchilla et al. 2007). Defences vary significantly between species, between individuals and between plant parts (Freeland & Janzen 1974). A range of plant secondary metabolites have been shown to play a role in determining intake by mammalian herbivores (Freeland & Janzen 1974; Wiggins et al. 2006). Plant secondary metabolites work to reduce the likelihood or duration of predation (Iason 2005); they influence herbivore behaviour, growth or survival through their repellent or

toxic nature or through reducing digestibility of plant material. The detoxification of plant secondary metabolites has been shown to influence the diet breadth of mammalian herbivores. Animals can avoid the negative consequences of eating too much of any one particular type of secondary compound by consuming a broad and polyphagous diet (Dearing & Cork 1999). Plant secondary metabolites have long been considered to be important regulators of feeding and dietary niche in vertebrates, but it remains a challenge to scale laboratory findings to the field (Foley & Moore 2005). In particular, the types and concentrations of plant secondary compounds are particularly variable because the combination and concentration of these compounds is often unique not just to a species, but sometimes to the individual plant (DeGabriel et al. 2014; Freeland and Janzen 1974).

1.5 Foraging theory

Foraging is generally understood using optimality models (Emlen 1966; MacArthur & Pianka 1966) which consist of a decision, a currency and a set of constraint assumptions: the costs and benefits of various foraging decisions are traded off to maximise net nutritional benefits (Stephens et al. 2007). To evaluate costs and benefits the key idea of 'profitability' was incorporated into in foraging theory. In particular, economic models have been applied to explain decisions about the choice of resources (diet width) and the optimal exploitation time of patches of resources separated in space e.g. the Marginal Value Theorem (Charnov 1976).

The development of this early, 'classical' foraging theory in the 1960s and 70s was biased towards carnivores and granivores because assumptions were made that when foraging optimally animals would be selected to maximise their net rate of *energy* gain over time i.e. energy gain is primarily what selection operates on (Emlen 1966). That is, when food items are of unequal nutritional value, the food providing the highest energetic profit (highest ratio of energy gains to losses) promotes fitness the most, because profit is defined in the currency of energy (Belovsky 1986). However, it has long been known that nitrogen is limiting in a variety of ecological systems (Mattson 1980; White 1993). Many organisms have a set daily intake requirement for nitrogen because it cannot be stored in the body, unlike energy. In particular, because plant foods provide a far smaller proportion of nitrogen to energy than animal tissues do, herbivores face very different foraging

decisions to those faced by carnivores and are likely to be strongly influenced by their daily nitrogen targets (e.g. Guglielmo et al. 1996; Joern & Behmer 1998). Energy gains associated with foraging decisions may not be as important as nitrogen gains in defining the 'profitability' associated with a herbivore foraging decision (Felton et al. 2009). This makes applying classical optimal foraging theory to understand nutritional strategies and foraging behaviour of herbivores more difficult (e.g. Westoby 1974; Owen-Smith & Novellie 1981; Senft et al. 1987).

Due to the multiple nutrient needs of herbivores and their existence in such heterogeneous, multidimensional and changing environments, classical optimal foraging models may lack the complexity to fully explain herbivore nutritional and foraging strategies. During the 1990s, a new dimension to foraging theory was developed, particularly by Simpson and Raubenheimer (1993) using a 'Geometric Framework' to conceptualise animal nutrition. The Geometric Framework Model was an important advance because it describes optimal foraging as simultaneously balancing multiple nutrient acquisitions when feeding on a multi-species diet, variable in nutrient content. This is a more realistic depiction of the environment encountered by the majority of herbivores than the classical net energy intake approach in optimal foraging theory. The Geometric Framework is a state-space approach focussing on a 'nutritional space' with multiple dimensions, each axis representing a functionally important (fitness-affecting) nutrient. Foods can be represented in the nutritional space using their nutrient ratios. An assumption of the model is that for an animal there is a point in space representing the optimal mix and proportion of these nutrients referred to as the 'nutritional target' and that foragers would have evolved a suit of physiological and behavioural traits which enable them to reach this target (Simpson & Raubenheimer 1995). Importantly, the nutritional target is dynamic and may change with life-stage or with changing environmental conditions. The Geometric Framework represents a potentially unifying conceptual framework for the field of nutritional ecology (Simpson & Raubenheimer 2012) and has the potential to increase significantly our understanding of nutritional strategies and foraging, particularly of polyphagous herbivores (DeGabriel et al. 2014).

1.6 A multi-faceted approach/key questions

In trying to understand aspects of nutritional ecology, previous studies have tended to be either very holistic e.g. wild vertebrates studied in the field (Rothman et al. 2011) or very reductionist e.g. animals studied in captivity in the lab (Abisgold et al. 1994; Behmer et al. 1998). However, this dichotomous approach in itself may be limiting. In order to increase understanding in this field I will use a multifaceted approach that integrates laboratory studies with landscape-scale field studies, therefore combining both holistic and reductionist approaches.

1.7 Chapter summaries

In this thesis I address the question: how do the nutritional strategies of brushtail possums enable them to obtain a balance of nutrients at native forest-pasture margins in New Zealand? This thesis comprises four data chapters that explore diet composition, diet composition in relation to nutrient contents of foods, intake rates of nutrients and landscape scale foraging behaviour of the common brushtail possum (*Trichosurus vulpecula*) in New Zealand. Each chapter begins with hypotheses and a brief justification for them. Finally, an overall discussion synthesises all these elements. Chapter contents are summarised below.

1.7.1 Chapter 2: The study species and study sites.

The study species is described, with particular regard to what is known about its nutritional ecology and the merits of using possums as a model study species. The remaining gaps in the possum-specific science are also described.

Two study sites are described from which the stomach and leaf samples were taken for results presented in Chapters 3 and 4.The site at which the GPS telemetry study in Chapter 6 was carried out is also described.

1.7.2 Chapter 3: Seasonal and sex variation in the foraging strategies of an alien invader: the brushtail possum in New Zealand.

The rationale for this chapter is that, understanding possum foraging strategies in their introduced range is particularly important. A detailed description of the diet composition at forest pasture margins, using methodology that can readily and

equally quantify leaf and non-leaf foods, is currently lacking. The aim is to understand how sex and season may influence foraging strategies, by describing how male and female possums forage and comprise their diets in a seasonally-specific way in order to meet their nutritional requirements.

In this chapter diets of wild male and female possums are described, in spring, summer, autumn and winter, to address the following questions:

- Does the diet composition of possums at forest-pasture margins vary with season or between males and females?
- How do possums use foods relative to their availability in the landscape?
- How important are non-foliar foods in possum diet composition?
- Is there seasonal variation in foraging behaviour, i.e. diet width, food importance, feeding strategy or niche breadth?

1.7.3 Chapter 4: Seasonal variation in nutritional content of plant foods available to brushtail possums at forest-pasture margins in New Zealand.

After describing the diet and feeding strategies of possums in Chapter 3, this chapter aims to describe the nutritional characteristics of potential foods. The rational for this chapter is that despite it being regularly predicted that possums must prefer high quality foods there has been very little study into the chemical composition of potential possums foods, therefore the influence of food quality on foraging strategies in New Zealand is currently incomplete.

In this chapter the nitrogen, available nitrogen, soluble energy and digestible energy content of potential foods is considered, using the 'avail-N' assay which integrates measures of browse quality to explain patterns in possum consumption. The following questions are addressed;

- Do potential foods vary in terms of any of the measures of nutrient content?
- How do flowers compare to foliage in terms of their nutrient contents?
- Do individual food types vary seasonally in any measures of their nutrient content?

- Are foods that have higher nutrient contents consumed more frequently or more intensively by possums?

1.7.4 *Chapter 5: Diet composition and nutrient intake rates of brushtail possums fed mixed diets.*

The rationale of this chapter is, to provide evidence from controlled laboratory studies that may help to explain some of the foraging strategies observed in possums at forest-pasture margins. Some seemingly high quality foods were not found in possums stomach contents. In particular, the use of supplementary foods, and the nutritional benefit that possums may gain from them, is not well understood. The nutritional quality of foods offered in a captive feeding trial are characterised in the same way as in Chapter 4. The objective was to determine the nutrient intake rates of possums fed three different diets; a leaf only diet, a leaf diet supplemented with flowers and a leaf diet supplemented with pasture. The following questions were addressed:

- Do possums vary the relative contributions of different species in the leaf diet when offered supplementary foods?
- Does the intake rate of dry matter, digestible matter or available nitrogen vary according to diet?
- Do possums have a similar target intake ratio of available nitrogen to energy, regardless of diet?

1.7.5 Chapter 6: Foraging pathway characteristics and habitat selection of the brushtail possum at forest-pasture margins in New Zealand.

The rationale for this chapter is to further understand the significant seasonal variation in the diet composition and foraging strategies of possums at forest – pasture margins. The aim is to characterise foraging pathways and determine the influence of possum sex or season on their characteristics. Furthermore, defining habitat selection by possums is critical to identifying the key foraging habitats for possums. Movement is studied at a fine enough resolution to divide foraging

pathways into two behavioural states: searching within patches and travelling between patches. The selection behaviour of possums can then be defined for each behavioural state separately and this information is used to address the following questions:

- Do males and female possums differ in the total time spent active, total distance travelled per night, proportion of time spent searching within patches and speed of travel between patches?
- Do those same possum foraging pathway characteristics vary between seasons?
- Do possums display selection for particular habitat types within the agricultural landscape?
- Does the nature of this selection vary between two different behavioural states, i.e. searching within patches and travelling between them?

1.7.6 Chapter 7: Discussion

In the overall discussion, elements from both holistic and reductionist approaches are synthesised and lab and field studies are combined to address the broad conceptual questions posed in Chapter 1. The possible significance of the findings in this thesis are then reflected upon in relation to the applied problem posed by this invasive alien pest, in terms of impacts on native forests and its potential as a vector for *Mycobacterium bovis* (Bovine Tb). Useful future work is also summarised.

Chapter 2: The study species and study sites

2.1 Study species: *Trichosurus vulpecula*

2.1.1 Possums as alien pest species

Worldwide, incidences and impacts of biological invasions are increasing (Ricciardi et al. 2000); they threaten biodiversity, ecosystem functioning, resource availability, national economies and human health (Vitousek et al. 1996; Vitousek et al. 1997). Globally, invasive species are considered the second largest threat to biodiversity after habitat loss (Park 2004). Since human colonisation of New Zealand/*Aotearoa* (first from Polynesia in 13th C and then Europe in 18th C) the introduction of a multitude of non-native species has had a significant and negative impact on biodiversity. Today, 22 terrestrial mammal species have established successful wild populations, to the detriment of many native species (Wodzicki & Wright 1984; King 1985; Wilson 2004). Furthermore, due to the extreme temporal and spatial isolation of the New Zealand landmass, levels of endemism and vulnerability to invasions are particularly high. As a result, the impacts of biological invasions in New Zealand are of high conservation concern, both in New Zealand and worldwide.

The Australian common brushtail possum (*Trichosurus vulpecula*) (hereafter referred to as the 'possum'), is arguably the most problematic of the suite of introduced species currently in New Zealand (Cowan & Tyndale-Biscoe 1990). Possums were introduced purposefully from their native Australia to Zealand in the late 19th C so that New Zealand could establish a fur trade with Europe (Clout & Ericksen 2000). This proved to be extremely successful: over the following 150 years, possums spread across New Zealand, establishing wild populations in every major habitat type, from remote expanses of native forest (including World Heritage designated sites) to remnant forest patches to native tussock grasslands and urban environments (Pracy 1974; Efford 2000). Realization that possums were negatively impacting biodiversity only began in the 1940s (Kean & Pracy 1953). Peak possum densities reached over 60 million in the late 20th C. Today, possums occur in over 95 % of New Zealand's land area, and are considered serious economic and environmental pests in all habitat types (Clout & Ericksen 2000).

2.1.2 Possum biology and ecology

T. vulpecula has a broad geographic range and occupies a wide range of different habitat types in its native Australia (Kerle 2001). This high degree of ecological flexibility has allowed possums to occupy a wide range of markedly different habitats following their introduction to New Zealand (Cowan 1990b). Estimates of population densities vary between 0.1 - 4.0 ha^{-1} in Australia (Kerle 2001) but New Zealand experiences the highest densities (e.g. 8.5 ha^{-1} in native broadleaf-podocarp forest). Some studies have recorded densities of over 30 ha^{-1} (Brown & Sherley 2002).

Possums are fecund and long lived species (Fletcher & Selwood 2000); they can live up to 15 years and reach maturity at one to two years of age (Green 1984; Cowan 1990b; Fletcher & Selwood 2000). Females are polyoestrous and pregnancy is short (*c.* 18 days). A peak in births occurs during autumn, and sometimes a second peak in spring if females have failed to breed previously or lost young, or are 'double breeding' females (Fletcher & Selwood 2000).

Possums are primarily solitary although their home ranges can overlap extensively; social interactions are normally limited to the breeding season and interactions between mothers and offspring (Day et al. 2000). In common with the majority of marsupials, *T.vulpecula* is nocturnal, spending the daylight hours denning above ground in epiphytes and tree hollows or on the ground underneath logs, roots or dense vegetation (Kerle 2001). Emergence from den sites is usually during the two hours after sunset, individuals returning to a den just before sunrise in summer, but often several hours earlier in winter (Nugent et al. 2000). In Australia feeding was observed in 1-3 bouts, with 2-3 hours in between (MacLennan 1984). However, Ward (1978) observed possums in the Orongorongo Valley in New Zealand and observed a bimodal pattern of feeding, with the first bout in the second hour after sunset and the second in the 6^{th} or 7^{th} hour after sunset.

T. vulpecula is a hind gut fermenting herbivore, with a simple stomach but extensive and well developed caecum and colon where foods undergo extended periods of microbial fermentation (Hume 1999). Long mean retention times of approximately 50 hours have been recorded (Foley & Hume 1987a; Sakaguchi & Hume 1990; McDowell et al. 2005). Some primarily-folivorous possums have a

highly specialised mechanism which allows them to selectively retain some particles in the hind gut for extended digestion whilst clearing indigestible fibre and other larger particles more quickly. However, a number of studies have found no evidence for this mechanism in *T. vulpecula* (e.g. McDowell et al. 2005*)* despite its presence in other similar taxa (e.g. rabbits; Sakaguchi & Hume 1990) and in other closely related arboreal marsupials (Wellard & Hume 1981; Foley & Hume 1987a). It is thought that the presence of this mechanism allows other small folivore species to consume a leaf only-diet, but without it, brushtail possums must supplement a leaf diet with other foods that are either highly digestible (low fibre) or have high nutrient concentrations. Physiological research has shown that *T. vulpecula* is unable to obtain the nutrients and energy required for survival, growth and reproduction through ingesting a leaf only diet (Kerle 2001).

2.1.3 Possum diet and foraging strategies

Possums are polyphagous; the diet in both New Zealand and Australia comprise largely foliage, but flowers, buds, fruits, pollens, saps, herbs, are also eaten regularly (Nugent et al. 2000). Due to their high levels of consumption of flowers, fruits and other non-leaf foods they have been described as 'reluctant' folivores' in New Zealand (Nugent et al. 2000). Common brushtail possums are also described regularly as 'opportunistic': they make heavy use of seasonally available, high-quality foods (e.g. fruiting trees) and may travel large distances to do so (e.g. Jolly 1976). Possums were described originally as pure herbivores, but there is now a large body of evidence that they also regularly eat animal material such as invertebrates and the eggs, nestlings and adults of native bird species, although this is often a relatively small proportion of the total diet (Clout 1977; Owen & Norton 1995; Rickard 1996).

In New Zealand, possums occupy a wide range of different habitats. Diet and food preferences vary substantially between habitats (Table 2.1). Over 100 native plant species have been recorded as being eaten in some locations, but the majority of possum feeding in any one place tends to be concentrated on a small subset of plant species; common forage species are described in more detail in Table 2.1. Possums have been consistently shown to be highly selective in their diet, and do not select foods based on availability (e.g. Cochrane et al. 2003); relatively

uncommon species often make up a large proportion of possum diet. There is significant spatial variation in which species are ‘preferred’ by possums (see Nugent et al. 2000). Preference rankings for food and their importance in the diet are not always consistent because availability and selectivity of feeding influence the preference index in any one location (Nugent et al. 2000).

Table 2.1: Summary of major possum diet studies in New Zealand, from Nugent et al. (2000). The eight most dominant foods reported in each study in terms of percentages of total dietary composition are listed. For those studies marked with asterisks the percentages are for the leaf component of diet only, whereas the others include fruits as well. Data are directly measured percent dry weights or calculated equivalents, except for the two earliest Orongorongo studies where the data are percentages of the total number (across all species).

	1	2	3	4	5	6	7	8
Silver beech forest S. Westland Owen & Norton 1995	*Aristotelia serrata* 31%	*Muehlenbeckia australis* 24%	Insect larvae 8%	*Rubus spp.* 5%	*Fuchsia excorticata* 4%	*Pseudopanax spp.* 4%	*Neomyrtus pedunculata* 3%	Fruit 2%
Silver beech forest S. Westland (Invading) Sweetapple unpubl.	*Muehlenbeckia australis* 21%	*Aristotelia serrata* 18%	*Weinmannia racemosa* 13%	*Fuchsia excorticata* 8%	Ferns 8%	*Peraxilla colensoi* 4%	*Rubus spp.* 3%	*Pennantia corymbosa* 1%
Silver beech forest S. Westland (Peak) Sweetapple unpubl.	*Fuchsia excorticata* 40%	*Aristotelia serrata* 13%	*Muehlenbeckia australis* 12%	Ferns 9%	*Weinmannia racemosa* 7%	*Raukaua simplex* 4%	Herbs 3%	*Melicytus ramiflorus* 2%
Silver beech forest S. Westland (Post-peak) Sweetapple unpubl.	*Weinmannia racemosa* 21%	Ferns 10%	*Fuchsia excorticata* 9%	*Aristotelia serrata* 9%	*Coprosma foetidissima* 7%	*Muehlenbeckia australis* 5%	*Melicytus ramiflorus* 5%	*Hoheria glabrata* 3%
Podocarp-hardwood forest, Stewart Island. March 1993. Nugent unpubl.	*Podocarpus hallii* 24%	*Metrosideros umbellata* 20%	*Pseudopanax simplex* 18%	*Senecio reinoldii* 8%	Ferns 7%	*Prumnopitys ferruginea* 4%	Bird 3%	Fungi 1%
Mixed hardwood-pasture Mt Bryan O'Lynn, Westland. Coleman et al. 1985	*Weinmannia racemosa* 33%	*Metrosideros umbellata* 24%	*Melicytus ramiflorus* 12%	*Pseudopanax spp.* 4%	*Coprosma foetidissima* 3%	Ferns 2%	Herbs and grasses 2%	*Raukaua simplex* 2%
Hardwood forest Alex Knob, Westland. Fitzgerald & Wardle 1979*	*Weinmannia racemosa* 27%	Ferns 16%	*Melicytus ramiflorus* 12%	*Metrosideros umbellata* 12%	*Fuchsia excorticata* 8%	*Schefflera digitata* 6%	*Ripogonum scandens* 4%	Unidentified sp. 3%
Hardwood forest Douglas track, Westland Fitzgerald & Wardle 1979*	*Metrosideros umbellata* 20%	*Weinmannia racemosa* 13%	*Schefflera digitata* 13%	*Fuchsia excorticata* 13%	Ferns 10%	*Melicytus ramiflorus* 10%	*Hoheria glabrata* 6%	Unidentified sp. 4%

Table 2.1 (page 2)	1	2	3	4	5	6	7	8
Podocarp-hardwood forest remnant Banks Peninsula, 1993 Suisted unpubl.	*Podocarpus hallii* 29%	*Fuchsia excorticata* 27%	Herbs 12%	*Rubus spp.* 8%	*Griselinia littoralis* 5%	*Muehlenbeckia australis* 4%	*Pittosporum eugenioides* 4%	*Pennantia corymbosa* 3%
Mahoe older forest remnant Banks peninsula. O'Cain 1997	*Myoporum laetum* 24%	*Muehlenbeckia australis* 20%	*Sambucus nigra* 14%	*Melicytus ramiflorus* 11%	*Solanum aviculare* 9%	*Cytisus scoparius* 7%	*Rubus fruticosus* 4%	Herbs 3%
Mixed beech podocarp Orongorongo, Wellington 1946-47. Mason 1958*	*Fuchsia excorticata* 14%	*Metrosideros robusta* 13%	*Alectryon excelsus* 12%	*Weinmannia racemosa* 9%	*Pseudopanax arboreus* 9%	*Brachyglottis repanda* 4%	*Leptospermum spp.* 4%	*Metrosideros fulgens* 2%
Mixed beech podocarp Orongorongo, Wellington 1967-73. Fitzgerald 1976*	*Weinmannia racemosa* 33%	*Metrosideros robusta* 29%	*Metrosideros fulgens* 9%	*Melicytus ramiflorus* 8%	*Ripogonum scandens* 7%	*Geniostoma legustrifolium* 5%	*Pseudopanax arboreus* 5%	*Elaeocarpus dentatus* 2%
Mixed beech podocarp Orongorongo, Wellington 1976-89. Allen et al. 1997*	*Metrosideros robusta* 36%	*Ripogonum scandens* 20%	*Weinmannia racemosa* 16%	*Melicytus ramiflorus* 16%	*Metrosideros fulgens* 6%	*Geniostoma legustrifolium* 3%	*Laurelia novaezelandia* 2%	*Elaeocarpus dentatus* 1%
Podocarp-hardwood Hihitahi, Central N.I. Rogers 1997	*Aristotelia serrata* 21%	Ferns 20%	*Podocarpus hallii* 17%	Herbs 9%	*Libcedrus bidwillii* 8%	*Carpodetus serratus* 6%	*Rubus spp.* 6%	Insects 2%
Podocarp-hardwood Waihaha, W. Taupo (pre-control) Nugent et al. 1997	*Podocarpus hallii* 19%	*Weinmannia racemosa* 18%	*Myrsine salicina* 10%	*Prumnopitys ferruginea* 8%	*Rubus spp.* 4%	*Alaeocarpus hookerianus* 4%	*Carpodetus serratus* 4%	*Aristotelia serrata* 3%
Podocarp-hardwood Waihaha, W. Taupo (post control). Nugent unpubl.*	*Rubus spp.* 17%	*Alaeocarpus hookerianus* 14%	*Podocarpus hallii* 9%	Fly larvae 7%	*Aristotelia serrata* 7%	*Muehlenbeckia australis* 7%	*Weinmannia racemosa* 4%	*Myrsine salicina* 4%
Podocarp-hardwood Pureora, Central N.I. Leathwick et al. 1983*	*Weinmannia racemosa* 20%	*Beischmieda tawa* 10%	*Melicytus ramiflorus* 9%	*Pseudopanax arboreus* 8%	*Pseudopanax edgerleyi* 7%	Ferns 6%	*Ripogonum scandens* 6%	*Rubus spp.* 6%

Table 2.1 (page 3)	1	2	3	4	5	6	7	8
Podocarp-hardwood Mokau, Northland. Suisted unpubl.	*Dysonxylum spectabile* 21%	*Alaeocarpus dentatus* 11%	*Rophalosylis sapida* 9%	*Weinnemania silvícola* 8%	*Metrosideros robusta* 7%	*Pinus radiata* 5%	Ferns **4%**	Herbs 3%
Tawa-hardwood Mapara, Central N.I. Leathwick et al. 1983*	*Beilschmiedia tawa* 24%	*Melicytus ramiflorus* 18%	*Alectryon excelsus* 14%	Ferns 13%	*Fuchsia excorticata* 7%	*Aristotelia serrata* 6%	Grasses 5%	*Geniostoma ligustrifolium* 3%
Kamahi-tawa forest, Mamaku Plateau, Central N.I. Clout 1977	Dicotolydons 68%	Fruit 10%	Ferns 4%	Flowers 3%	Monocotyledon 3%	Invertebrates 2%	Bark <1%	*Pinus radiata* <1%
Pohutukawa Rangitoto Island, Auckland. Olds 1987*	*Astelia banksii* 23%	*Myrsine australis* 21%	*Metrosideros spp.* 95%	*Cyathodes juniperina* 13%	*Griselinia lucidia* 7%	*Coprosma spp.* 6%	*Coriaria arborea* 2%	*Trichomanes reniformes* 2%
Alpine shrubland Rangitata, Central S.I. Parkes & Thomson 1995	*Podocarpus nivalis* 22%	*Muehlenbeckia axillaris* 11%	*Coriaria angustissima* 10%	*Hieracium spp.* 8%	*Hoheria lyallii* 6%	*Trifolium repens* 5%	*Raoulia tenuicaulis* 4%	*Aristotelia fruticosa* 3%
Pine plantation Tokuroa, Central N.I. Clout 1977	Dicotyledons 45%	*Pinus radiata* 31%	Ferns 9%	Invertebrates 2%	Monocotyledon 1%	Flowers 1%	Fruit 1%	Bark <1%
Pine-plantation – Tokoroa, Central N.I. Clout 1977	Dicotyledons 40%	Monocotyledon 30%	Ferns 14%	Fruit 2%	*Pinus radiata* 1%	Bark <1%	Flowers <1%	Invertebrates <1%
Pine plantation Ashley Forest, Canterbury. Warburton 1978	*Cystisus scoparius* 27%	*Pinus radiata* 22%	Grasses 12%	*Rubus fruticosa* 11%	*Ulex europaeus* 4%	*Muehlenbeckia australis* 3%	*Rumex acetosa* 3%	*Pseudotsuga menziesii* 2%

2.1.4 Possum impacts and control in New Zealand

Possums negatively impact biodiversity in New Zealand in a number of ways: through consuming foliage they can defoliate and kill individual trees, which can lead to whole canopy-collapse in vulnerable forest types (Rogers & Leathwick 1997). Possum feeding preferences for particular tree species can alter vegetation composition over time, which subsequently has knock-on effects throughout the ecosystem. In addition, the dietary flexibility of possums means they are able to switch to less preferred species if and when their most preferred foods are completely removed from an area (Nugent et al. 2000). By consuming fruits and flowers possums can suppress or change the reproductive output of plants and can alter patterns of seed dispersal, seedling establishment, forest regeneration and succession. Impacts on populations and communities of native fauna can be direct (e.g. predation of eggs and nestlings of endemic bird species) or indirect (e.g. resource competition and dietary overlap with native species). Possums and have been implicated in the decline of several native bird species (James & Clout 1996; Wilson 2004).

In addition, possums are both a risk to human health and costly to New Zealand's economy because they are the primary vector of bovine tuberculosis (Tb) in livestock. As a result, possums are subject to widespread and ongoing control operations throughout New Zealand (over thousands of hectares annually) at a substantial economic cost. In 2000, the NZ government released figures that show possum control requires an ongoing annual expenditure of $42.7 m by the Crown and $74.8 m by the private sector. Further direct economic losses to the private sector were estimated to be as high as $60m per year (NZ Parliamentary Library 2000).

Possum control in New Zealand is coordinated by three main groups, each with a different remit: the Animal Health Board is concerned with minimising the Tb risk posed by possums to domestic stock; the Department of Conservation aims to protect native flora, fauna and protected areas from possum damage; and the Regional Councils are responsible for both the conservation of indigenous biodiversity and Tb control. To break the Tb transmission cycle, possum

populations must be maintained at between 5 % and 25 % of carrying capacity of most forest habitats (Coleman & Livingston 2000). This is achieved through an initial control operation followed by annual or biannual control, particularly focussed on forest-pasture margins where population densities are particularly high (Coleman & Livingston 2000). Possums have successfully been eradicated from several offshore islands (Brown & Sherley 2002; Mowbray 2002), and some 'mainland islands' (Lovegrove et al. 2002). However, in most areas of New Zealand the current management objective is sustained maintenance of low density populations.

Chemical poisoning is the most effective current method of possum control. Large and inaccessible areas of are most often treated with aerial applications of the poison '1080' (often as part of an integrated approach that also targets rodents, mustelids and feral cats). In smaller areas, ground-based chemical control is carried out using a range of toxins, including 1080, cyanide, cholecalciferol and various anticoagulants (e.g. Brodifacoum). Poisoning operations can have substantial detrimental impacts through non-target and secondary poisoning (Parliamentary Commissioner for the Environment 1994), particularly native bird (Eason et al. 2001), some of which are endemic and critically endangered (e.g. NZ Saddleback, Prof. Ian Jamieson *pers. comm*.). Chemical control is regularly supported and followed by additional, physical control methods such as trapping and shooting (Cowan 2005). A combination of control methods is often required for long-term possum control or to achieve total eradication of possums from an area. On Rangitoto and Motutapu islands an aerial drop of the poison '1080' was estimated to kill 93% of the population over a few weeks, but it took a further 9 years of physical control using leg-hold traps and ground-based shooting to eradicate possums entirely (Mowbray 2002).

2.1.5 *Possums as a model species for increasing our understanding of nutritional ecology*

T. vulpecula is particularly suitable model species for increasing our understanding of herbivore nutritional ecology for a number of reasons. Destructive sampling of possums in New Zealand is encouraged, which allows for relatively easy monitoring of diet at an individual level through analysis of stomach contents,

without the need for direct observations. The identity and relative proportions of different food species in possum diets can be assessed accurately using the stomach layer separation technique. Furthermore, possums are widespread across New Zealand so can be trapped or studied easily in the wild. Possums are nocturnal and forage in dense, multilayer canopies, which make direct observations of foraging very difficult. Instead, possum movements can be observed more easily in an indirect way, by using GPS telemetry techniques. Adult brushtail possums are large enough that they are capable of carrying GPS/VHF tracking devices that collect spatiotemporal location data at set time intervals.

At the other end of the spectrum, experimental approaches require studying the model species in captivity under controlled laboratory conditions; for the majority of wild vertebrate herbivores this is impossible. However, in New Zealand possums present an opportunity in that they can be kept and studied in captivity in under animal ethics board control.

2.1.6 Opportunities for future research into the nutritional ecology of T. vulpecula in New Zealand

In New Zealand, possum browsing pressure on native plant species, predation on native birds and invertebrates resulting in negative impacts on native ecosystems are well documented. However, measures of the relative quality of different food items consumed by possums and an understanding of how food chemistry influences food preference and foraging behaviour in New Zealand is lacking. The majority of possum feeding in any one location tends to be concentrated on a relatively small subset of plant species with significant spatial variation in what species are ‘preferred’ by possums (Nugent et al. 2000). Preference implies a behavioural and nutritional basis to foraging decisions, but these have not been adequately explored. The nutritional composition of New Zealand plant species has very rarely been quantified to explain patterns in possum consumption. It has been assumed widely that possums select ‘high quality’ foods, but this remains largely speculative, despite a strong theoretical basis for supposing a link between food quality and reproductive success.

It can be argued that nutritional ecology research is fundamentally interdisciplinary (Raubenheimer et al. 2009; Simpson & Raubenheimer 2012) and therefore, when evaluating foraging decisions, a similarly multifaceted approach should be used. Many previous studies have tended to use either very reductionist (artificial laboratory-based experiments) or very holistic (field observations of wild vertebrates) methodological approaches to answering research questions. However, this research aims to integrate multiple approaches using the possums as model species that permits a research approach that combines highly controlled laboratory studies of captive animals with field observations of wild conspecifics.

2.2 Study Sites: New Zealand native forest-pasture margins

2.2.1 The wider landscape

This research was conducted within a mixed farmland-forest-scrub landscape near the city of Dunedin on the south island of New Zealand's east coast (-45.871738, 170.503086). The topography of the study region is dominated by a series of volcanic outcrops and peaks, including Mihiwaka (561m), Mount Holmes (575 m) and Mount Cargill itself (676 m) which descend to Port Chalmers and the Otago Peninsular coast to the east and Waitati inlet to the north. Before human settlement, indigenous broadleaf-podocarp forest was the dominant vegetation cover in the study region. Today, exotic grassland is the primary vegetation cover; only secondary-growth native forest and native scrub remain and comprise only a relatively small proportion of the land area.

The wider area comprises a fragmented rural landscape: land cover is dominated by exotic grassland interspersed with remnant patches of secondary native forest and scrub (Figure 2.1). Primarily the land use is grazing (especially cattle, sheep and horses), but exotic forestry plantations are also important. Some of the remaining indigenous vegetation is located on private land, sometimes under protection from land covenants. The remainder comprises a network of protected areas: the 'Hill Reserves', owned and managed by Dunedin City Council; Graham's Bush Scenic Reserve, owned and managed by the New Zealand Department of Conservation; and Orokonui Ecosanctuary (250 ha), a fenced reserve where predators and non-native species are permanently excluded.

2.2.2 Stomach and vegetation sampling sites – 300 and 353 Mount Cargill Road

Two adjacent farms comprised the site used for stomach sampling and subsequent collection of dietary items. The farms were 10 km north of Dunedin, off opposite sides of Mount Cargill Road, which passes north-south through the study region along a ridge at approximately 500 meters above sea level (Figure 2.2). At 300 Mount Cargill Road (45°80′S, 170°57′E) the Thom family farm several acres, primarily grazing sheep and highland cattle. Altitude at the study site ranges from

240 m to 340 m above sea level. The land is adjacent to Grahams Bush Scenic Reserve which occupies the valley to the south of the study site. Livestock are excluded from this reserve at all times. At 353 Mount Cargill Road The Morrison family and their neighbour Ian Fraser farm their land together, grazing horses, cattle and sheep on the west facing slopes of Mount Cargill (45°80′S, 170°57′E). Altitude at the study site ranges from 260 m to 380 m above sea level. On both farms, livestock graze pastures but also have periodic access to the forested and scrub areas. As a result, the understory and canopy can be altered significantly.

Both farms contain the same broad habitat types. Three tree habitats predominate: broadleaved indigenous hardwoods, dominated by secondary-growth shrub species (*Fuchsia excorticata*, *Melicytus ramiflorus*, *Aristotelia serrata*, *Griselinia littoralis*, *Myrsine australis, Pittosporum eugenioides*, *Carpodetus serratus*, *Schefflera digitata*, *Pseudopanax crassifolius*, *Pseudowintera colorata* and *Coprosma* spp.) with some areas containing podocarps (including *Prumnopitys ferruginea* and *Dacrydium cupressinum*); native kanuka/manuka bush (dominated by *Kunzea ericoides*) and exotic forest (pine plantations /macrocarpa shelter belts). Two broad grassland habitat types are present; primarily pasture (introduced spp.), but there are also areas of rough grassland. The final habitat type in the study site is non-native scrub and this consists of substantial areas of introduced gorse and broom.

2.2.3 GPS telemetry study site – Pigeon Flat

The study areas in which possums were trapped for stomach contents was separate from the site at which possum movement behaviour was studied using GPS telemetry. The Anderson family farm the land at Pigeon Flat primarily for grazing and raise cattle and pigs. The study site was centred upon a fenced remnant of native broadleaf-podocarp forest, within a wider native forest-pasture landscape, typical of farmland in lowland Otago (Figure 2.2). Patches of native secondary broadleaf forest and native shrub/scrubland occurred alongside patches of introduced scrub and grassland habitats (Figure 2.3). The telemetry study site was comprised of a mixture of 13 different habitat cover classes, which are described in detail in Chapter 6.

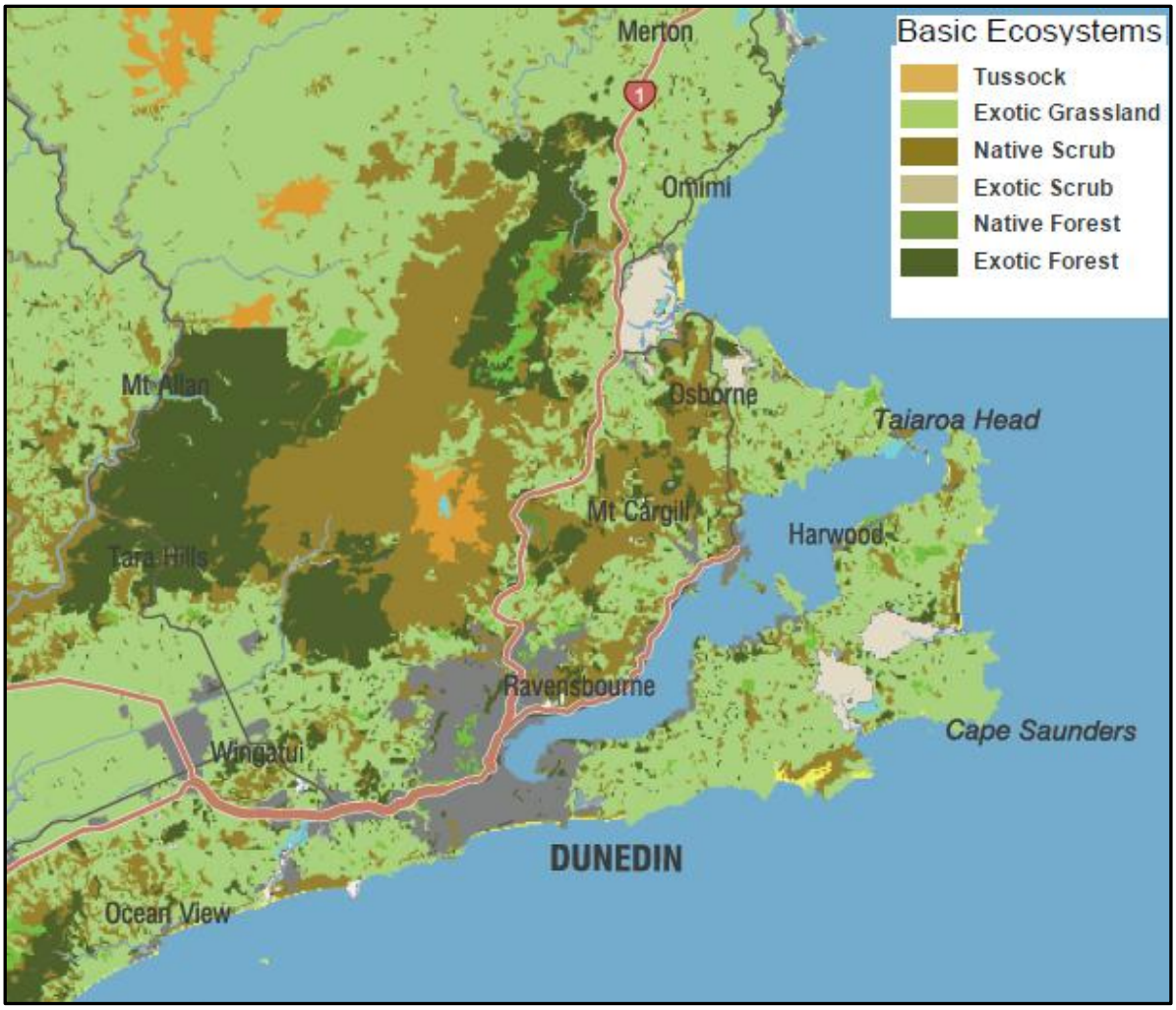

Figure 2.1: Vegetation cover classes over the study region and the wider area around Dunedin, South Island, New Zealand (The Land Cover Database, released in June 2014).

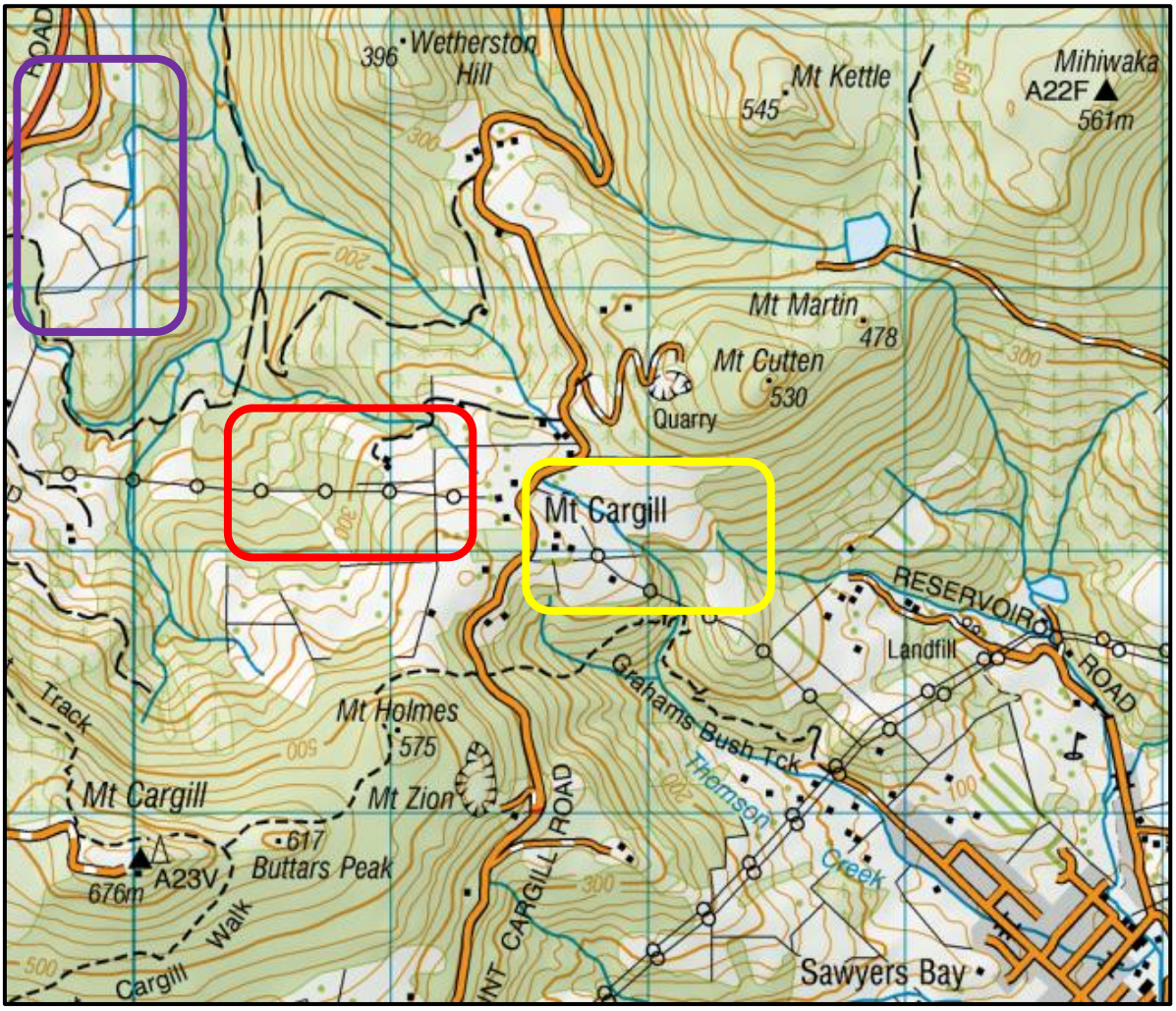

Figure 2.2: Locations of the study sites for stomach and vegetation sampling, showing the location of the farm at number 300 Mount Cargill Road (yellow) and 353 Mount Cargill Road (red). Approximate location of the site used for GPS telemetry sampling is also shown (purple). Topographic map from Land Information New Zealand (LINZ).

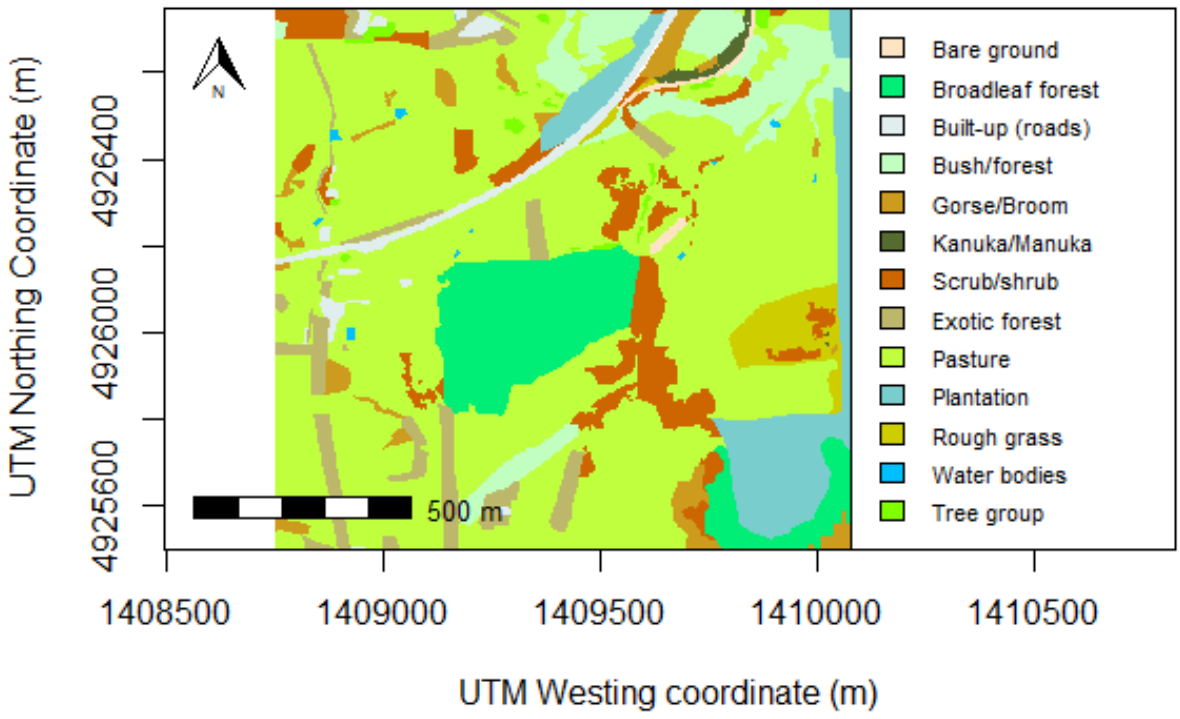

Figure 2.3: A habitat map of the telemetry study site at Pigeon Flat, Otago, New Zealand, showing the 13 different habitat cover classes. From vanHeezik (unpublished data).

Chapter 3: Seasonal and sex variation in the foraging strategies of an alien invader: the brushtail possum in New Zealand.

3.1 Abstract

Brushtail possums are a conservation pest in New Zealand where they have a widespread deleterious impact on indigenous ecosystems. Understanding possum foraging strategies in their introduced range is vitally important for both discerning and mitigating their impacts, and for improving the efficacy of the extensive and expensive control operations. Despite this, specifics of seasonal and sex variation in possum foraging strategies at forest-pasture margins are not currently known.

Eighty one possums were trapped in at forest-pasture margins within an agricultural landscape in New Zealand. Stomach contents were dissected following the layer separation technique, which permitted the separation and measurement of each single-species 'meals' consumed within a foraging night. The frequency of occurrence and the relative abundance of each food in the stomach contents were quantified. I found significant seasonal variation in the diet composition of possums but no differences between males and females. The annual diet was composed primarily of leaves of woody plants. The primary leaf diet was supplemented in all seasons; in autumn non-foliar foods comprised a higher proportion of the diet than leaves did. Flowers comprised 45% of the diet in spring and fruits 49% in autumn, while larval invertebrates occurred in 41 % of stomachs in winter and 35 % in spring.

A graphical analysis of stomach contents (Amundsen et al. 1996) revealed that possums had a polyphagous feeding strategy in all seasons but the importance of different foods in the diet varied with season. Possums had a broad niche width in all seasons, but there was a degree of specialisation on *K. ericoides* foliage in winter. Diet width was greatest in autumn when 14 species of food were eaten including a wide range of fruits and narrowest during winter and spring when seven food species were eaten.

I conclude that brushtail possums in New Zealand are opportunist feeders that threaten some species through their selective browsing, and that synchronising control measures to the proximity of the most preferred food plants in a season-specific way could increase efficiency of control measures, most effectively during winter when the highest proportion of the diet was comprised by a single most preferred species.

3.2 Introduction

3.2.1 Resource use and foraging strategies

Understanding resource use is fundamental to identifying factors that affect the distribution and abundance of organisms. Therefore it is of substantial theoretical and applied interest to ecologists, particularly in the context of biological invasions (Lohrer et al. 2000; Shea & Chesson 2002; Simberloff et al. 2013). Food resources are fundamentally important to all heterotrophic organisms which are unable to synthesise the organic compounds they need to survive, grow and reproduce. Furthermore, many organisms have multiple nutrient needs so must choose diets and obtain nutrients in a way that meets all of these different nutritional targets simultaneously to avoid negative fitness consequences (Simpson & Raubenheimer 1993). Foraging strategies can therefore be defined as a suite of tactics that relate to how animals acquire a balanced nutrient uptake from the range of different food resources in the environment (Stephens & Krebs 1987).

3.2.2 Seasonal and sex variation in foraging strategies

Foraging strategies that require the ingestion of a relatively large number of different food items (i.e. polyphagy) is a key behavioural mechanism through which herbivores can acquire an optimally nutritious diet. Their foraging environment is abiotically and biotically heterogeneous at a range of temporal and spatial scales. For herbivores, spatial and temporal variation in plant availability, quality and distribution mean they must adjust their diets to meet their nutritional requirements. As a result, seasonal variation in foraging habits is a common pattern among herbivores and is often linked to fluctuations in the quality and availability of particular food resources (van Langevelde & Prins 2008).

As well as differences in foraging strategies across the annual cycle, individuals within a species often display differences in their foraging strategies because of fundamental differences in nutrient requirements between age, sex or reproductive class. Therefore, the diet composition of male and female individuals may differ significantly, at least at some times of the year, in terms of the food types included and/or the amounts eaten relative to each other. Quantifying and comparing the relative importance of food types in the diet at different times of the year in male

and female animals can be used to infer seasonal and sex differences in foraging strategies and resource use patterns.

3.2.3 Measuring diet and the Amundsen approach

There are a number of ways of quantifying dietary composition. Two very common approaches include recording the Frequency of Occurrence (% FO) and relative quantity (by numeric abundance (% N), mass (% M) or volume (% V) of stomach contents) of food items in the diet (Hynes 1950; Hyslop 1980). This allows interpretation (mostly *via* personal judgement) of the relative importance and dominance of particular food types (Costello 1990). More recently, Amundsen et al. (1996) proposed a new parameter for the analysis of dietary composition: the *'prey-specific abundance'* (% PSA), defined as the percentage of the masses in only the stomachs in which the food type occurred, provides a prey-specific measure of feeding intensity on different prey types. Hence PSA can identify foods eaten in large amounts but by only a subset of the population. The various measures that can be derived from stomach contents analysis are useful for quantifying and identifying patterns in dietary composition and resource use and all are potentially informative in defining the key components of foraging strategies.

Recent work in the field of fish biology has developed our ability to analyse and deduce foraging strategies from the analysis of stomach contents data. This advance has yet to be applied to terrestrial herbivores. A graphical approach was first proposed by Costello (1990) and later modified by Amundsen et al. (1996), Fig 3.2. The approach allows the analysis of diet composition in respect to a number of dimensions (i.e. measures of 'use') simultaneously. The multifaceted approach provides an improved method through which to determine key foraging strategies for particular foods and patterns in resource use. Each food type is described and plotted in relation to two axes, % PSA and % FO and the resulting graph allows the analysis of food importance, feeding strategy and inter- and intra-individual components of niche breadth to be analysed (Amundsen et al. 1996; Deus & Petrere-Junior 2003). The interpretation of these factors can be obtained by examining the distribution of points along the diagonals and axes of the graph (see Figure 3.2 and methods section for more details).

The use of analysis of stomach contents is constrained as a technique for studying the diet composition and foraging strategies of mammalian herbivores for ethical reasons; it involves destructive sampling of a species not hunted under licence. Instead, direct observations of feeding animals and the marks they leave on predated plants are often used to monitor food intake of animals in the field. Diet can also be inferred from analysis of scats or other tissue (e.g. blood for stable isotope analysis). Invasive species that are subject to regular population control measures are an exception to this constraint. For such species, destructive sampling is appropriate and often encouraged (e.g. with bounties). This offers an unusually favourable opportunity for scientific studies of stomach contents of animals that have fed in their natural habitat and is particularly appropriate for herbivores with a high proportion of indigestible material in their diets.

3.2.4 Variation in possum foraging strategies

In New Zealand, introduced brushtail possums have been recorded to eat hundreds of different food items, but the majority of feeding in any one place is concentrated on only a small subset of the full range (Nugent et al. 2000). Variation in resource use is observed commonly in brushtail possums foraging in both their native and introduced ranges. For example, Cochrane et al. (2003) found significant seasonal variation in the relative abundance of foliar and non-foliar food types in possum diets in New Zealand beech (*Northofagus*) forest. Deciduous *Muehlenbeckia australis* foliage ranged between 2.9 and 27.8 % of the diet over the four sampling seasons and *Weinmannia racemosa* flowers comprised between 0 and 35.7 % in the same time period. Foliage from the main canopy *Northofagus* species comprised less than < 1% of the diet in all seasons. Seasonal trends in the use of evergreen foliage by possums have also been recorded and have been attributed to seasonal changes in the relative palatability of particular food types (Nugent et al. 2000), although the chemical composition of most foods is unknown.

Possum selection of particular species is likely to reflect their availability in the landscape. But in general it has been shown that some foods are consumed by possums in larger amounts than would be predicted by their availability alone. Dominant dietary constituents often have a limited spatial and temporal distribution. For this reason, a number of authors have concluded that possums

are ‘selective’ foragers (e.g. Cochrane et al. 2003) as is the case for most polyphagous species due to differences in nutrient composition of different food items and the dynamics of the physiological requirements of the animals. Preference or selection for particular food items, which can vary seasonally for the same plant species, implies specific nutritional drivers of dietary composition. However, aspects of dietary composition and foraging behaviour of possums at bush-pasture margins are not well understood.

3.2.5 Specific ‘gaps in the science’

There is a clear need to improve understanding of foraging strategies used by brushtail possums in their introduced range. Possum diet composition has not been studied in detail in secondary broadleaf-podocarp forest fragments and edges that are bordered by grazing pastures. This is despite the high conservation value of secondary forest in the many agriculturally-dominated regions of New Zealand that have been highly modified by humans and in which no primary forest cover remains. The extent to which possum rely on particular foods within this matrix is unclear which makes their potential impacts difficult to assess and means that control operations may be less efficient than they could be. Furthermore, where possums exist at high densities and close to livestock, the risk of Tb transmission is maximal so an increased understanding of possum foraging behaviour, through detailed analysis of patterns in dietary composition and foraging strategies, is very important in these areas.

There is a lack of understanding of how possums forage and comprise their diets in a seasonally-specific way in order to meet their nutritional requirements and the specifics of how resource use may vary between reproductive classes within the population. This constrains the ability to explain the particularly high possum densities recorded at bush-pasture margins in terms of the resources used and the ‘nutritional niche’ of possums. Such research could lead to key insights into possum foraging strategies and an improved understanding of the consequences of these traits for native flora and fauna and livestock.

3.2.6 Aims, objectives and hypotheses

This Chapter describes the dietary composition and foraging strategies of brushtail possums at secondary forest-pasture margins using quantitative stomach contents analysis. Diet is described for both males and females, in four seasons, in terms of its composition of individual food species, which were also grouped into six primary food types: tree foliage, scrub foliage, foliage of vines, flowers, fruits, and invertebrates. Three dietary measures are used (M, FO and PSA) to investigate variation in possum diet composition. Foraging strategies (namely; food importance, diet width, feeding strategy and niche width) are described using Amundsen's (1996) graphical approach.

The following hypotheses are tested:

- **H1:** In all seasons, possum use of individual food species will be limited to those species that occur frequently in the landscape.
- **H2:** The majority of possum diet will be comprised of leaves in all seasons.
- **H3:** Both season and sex will significantly influence the frequency of occurrence (FO) and relative abundance by mass (M) of food types in possum diet.
- **H4:** Foraging behaviour will be strongly seasonal; Diet width, food importance, feeding strategy and niche breadth will all vary with season.

3.3 Methods

3.3.1 Possum trapping and stomach sampling

Sampling took place in four different seasons over one annual plant-growth cycle; August 2012 (winter), October 2012 (spring), January 2013 (summer) and March 2013 (autumn). On each occasion, the stomach contents of 9-11 male and 9-11 female possums were analysed. The diets of 81 adult possums were analysed in total. Possums were trapped using Timms kill-traps placed along bush-secondary forest margins at two adjacent farms on the lower slopes of Mt Cargill (676 m), 15 km north of Dunedin in the South Island of New Zealand. The study sites have been described in detail in Chapter 2.

The availability of plant food items in the landscape was scored in terms of the species relative abundance. To measure abundance I used an estimate of the percentage cover and assigned the categories according to the percentages as follows: Dominant: >75% (no species were placed into this category so it was not used); Frequent: 40 – 75% - common over most of the site; Occasional: 10-39% - occurred regularly in several areas of the site; Rare: <10 % - occurred in small numbers and/or in only small areas of the study site.

Possum traps were placed at the base of trees and baited with apple. Cinnamon and icing sugar were spread at the base of the trap and up the trunk of the tree to act as a lure. Traps were checked each morning and dead possums were brought back to the laboratory where sex and mass were recorded. Pouch young were euthanized if present. To control for the effects of ontogenetic variation, only adult possums were used in the subsequent analyses.

Possums were dissected along their fronts from the sternum, down and across the side of the rib cage to allow removal of the entire stomach. Stomachs were weighed whole, and then frozen immediately for later dissection and determination of stomach contents.

3.3.2 Identification of species eaten

The ‘layer separation’ method described by Sweetapple and Nugent (1998) was followed. Possum stomachs were thawed and cut along their greater curvature to reveal their contents (Figure 3.1). Contents of the area immediately before the duodenum were excluded because in this part of the stomach, layers are subject to mixing and so do not retain their discrete form. Layers were separated using a small spoon and identified to species-level, where possible, using a dissecting microscope. Identification was aided by reference collections, assessing differences in texture, leaf vein pattern, colour and other aspects of appearance (Sweetapple & Nugent 1998). However, some items were identified only to broader categories e.g. invertebrates. Items such as small stones and fur-balls were presumed to have been consumed incidentally and not to have added to nutrients assimilated, so were excluded from the analyses. Where leaves and other plant tissues, such as fruits and flowers, were consumed from a single species simultaneously forming a mixed layer, the proportion of each type of plant tissue within the layer was estimated by eye. After identification, samples were placed on labelled metal trays and in a drying oven at approximately 70°C for 24 h until dried to constant weight. Finally, dried samples were weighed and the proportional contribution of each item to the total dry mass of stomach contents was calculated.

The number of stomachs in which each item occurred was recorded and expressed as a percentage of the total number of stomachs examined (% frequency of occurrence). A gravimetric measure of abundance was also obtained whereby each food item was expressed as a percentage of the total biomass in the stomach (% mass). Food remains comprising > 1 g wet weight of the stomach were grouped into six classes for some analyses. These were; tree foliage, scrub foliage, vine foliage, flowers, fruits and invertebrates.

Figure 3.1: Dissected intact stomach of a brushtail possum (photograph by J. Morley).

3.3.3 Calculations of diet composition descriptors

Diet composition was described and analysed according to three different measures:

i) Frequency of Occurrence (FO)

This was used to describe the level of 'use' of a particular food item in a population (Hyslop 1980). The method has been used frequently to illustrate seasonal and sex differences in the diet composition of polyphagous species (Amundsen et al. 1996).

FO_i (%) = (n_i/n)*100
Where n_i is the number of stomachs food item i occurred in, and n is the total number of stomachs analysed.

ii) Contribution to dry mass of stomach content (M)

This was used to determine the intensity of feeding on each food type. This measure of the relative abundance of a food type in the diet is often taken to represent the nutritional value of the food/prey (MacDonald & Green 1983; Cortez 1997).

M_i (%) = (M_i/ ΣM_n)*100

Where M_i is the dry mass of food item *i* in a unit (stomach) and M_n is the dry mass of all food items in a unit.

iii) Prey Specific Abundance (PSA)

The PSA variable was derived from the raw data. This measure is thought to improve on % M in that it measures abundance in only the stomachs containing that food type. Hence PSA can identify foods eaten in large amounts but by only a subset of the population e.g. females.

PSA_i (%) = (M_i / ΣM_{in})*100

Where M_i is the dry mass of food item *i* in a unit (stomach) and M_{in} is the dry mass of all food items in a unit that contains food type *i*.

3.3.4 Hypothesis testing

Hypothesis 1 was tested by determining the individual food species eaten by possums and comparing their use, to their availability (relative abundance) in the landscape. Hypothesis 2 was tested by examining the effect of season and sex on the frequency of occurrence (FO) and the relative abundance (M) of each of the broad food type categories. Hypothesis 3 was tested by assessing the species composition of possum diets in four different seasons in terms of M and FO. Hypothesis 4 was tested using the graphical approach developed by Amundsen et al. (1996), where foods were defined according to their frequency of occurrence (FO) and their prey specific abundance (PSA).

3.3.5 Statistical analyses

Stomachs were analysed from subsamples of 18-20 stomachs per season, 38-40 stomachs per sex and 9-11 stomachs per season/sex. The significance threshold used in this research was set at $p < 0.05$. All statistical analyses were performed in R 3.2.1.

Differences between seasons and between males and females in the proportion of the dry mass of each food type in stomach contents (% M) were tested using non-parametric Kruskal-Wallis tests. Differences between seasons and between males and females in the frequency of occurrence of each food type in stomach contents (% FO) were tested using Chi-square tests. In this case, the outcome variable was considered to be categorical i.e. a binary variable, because each possum either ate, or did not eat, the food type.

3.3.6 Graphical analyses

The graphical approach to assessing foraging strategies from stomach contents data (Amundsen et al. 1996) requires that each food is described and plotted in relation to two axes, % PSA and % FO. The interpretation of food importance, feeding strategy and niche breadth can be obtained by examining the distribution of points along the diagonals and axes of each graph (Figure 3.2) in the following ways:

I. *Food importance*: The diagonal from the lower left to the upper right corner provides a measure of food importance, with dominant foods at the top, and rare or unimportant foods at the lower end.

II. *Feeding strategy:* The vertical axis represents the feeding strategy of the predator in terms of specialization or polyphagy: predators have specialized on food positioned in the upper part of the graph, whereas food items positioned in the lower part have been eaten only occasionally (polyphagy). Food points located at the upper left of the diagram would be indicative of specialization by individual predators, and those in the upper right would represent specialization of the predator population.

III. *Niche breadth*: Food in the upper right are specialised upon by the whole population so one can infer a narrow niche breadth if most foods are located in the upper right of the diagram. Niche breadth can be interpreted in terms of both its intra-individual component (BPC) (i.e. different individuals specialising on different food types) and inter-individual component (WPC) (i.e. most individuals utilising many resource types simultaneously). Food in the upper left have high specific abundance and low occurrence so are consumed by a few individuals displaying specialisation. Food types in the lower right have a low abundance but high occurrences so are eaten occasionally by most individuals.

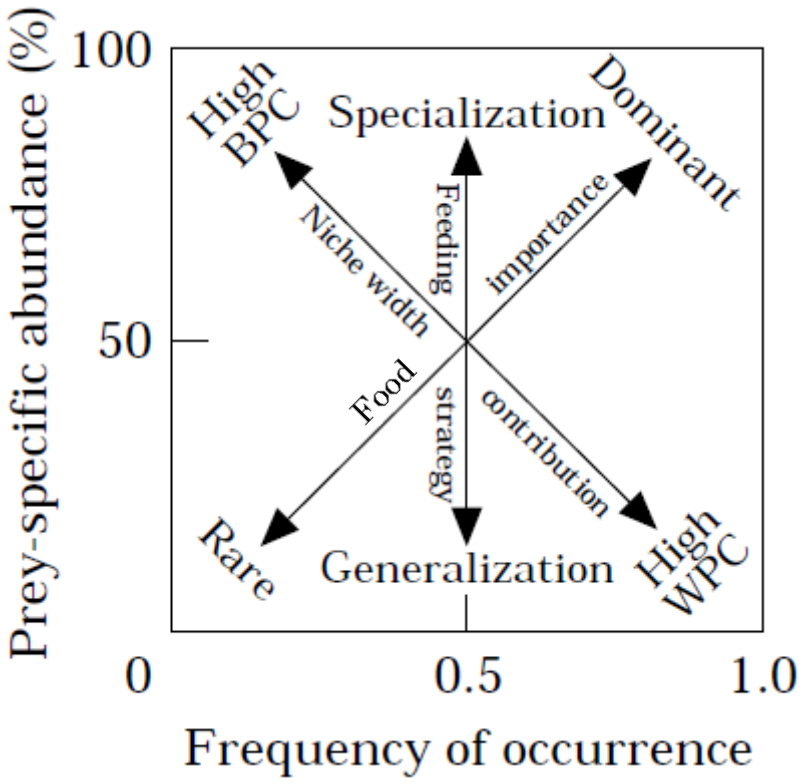

Figure 3.2: Explanatory diagram for the graphical interpretation of feeding strategy, niche width contribution and food importance from stomach contents. From Amundsen et al. (1996).

3.4 Results

3.4.1 Possum diet and availability of foods

Annual diet was dominated by the foliage of two soft-leaved, deciduous, seral species (*F. excorticata* and *M. australis*) and one non-deciduous shrub (*K. ericoides*) (Table 3.1). Seral vegetation tends to be dominated by species with deciduous or short-lived leaves (Opler et al. 1980). *F. excorticata* is a small tree (<12 m tall) which is common in damp forest margins and regenerating areas. It flowers from August to December then produces fleshy, purple berries. *M. australis* is a climbing plant common on edges and in regenerating habitat. Flowers of *M. australis* bloom from late spring to autumn. Fruits are present from November to April. *K. ericoides* is a hardwood shrub (<10m) which occurs commonly in scrubland and is known as a colonising plant which aids forest recovery from fire and other disturbance events. *K. ericoides* and *M. australis* both occurred frequently in the landscape at the study site but *F. excorticata* occurred only rarely (Table 3.1).

As well as foliage, fruits and flowers were ranked highly in terms of their contribution to the annual diet of possums (Table 3.1). Several tree, shrub and vine species were used as a source of flowers and/or fruits as well as foliage (Table 3.2). Conversely several tree species were used solely for their fruits and their foliage was not consumed (Table 3.2).

All but one of the species that possums used for foliage as well as fruits or flowers occurred frequently in the landscape (Table 3.2). With the exception of *F. excorticata*, dominant foliar foods were not rare trees (Table 3.2). *F. excorticata* was a rare tree but was an important food source from which possums took foliage, fruit and flowers (Table 3.2). The other rare tree used by possums was *C. serrata* which was used only for fruit consumption in autumn (Table 3.2).

As expected, some food items were unavailable in some seasons so were not consumed (e.g. flowers in autumn and fruits in winter and spring).

Table 3.1: Ranked importance of individual food items found in male and female possum stomachs, pooled across all seasons (according to the mean proportion of dry mass (M)). The relative abundance of food items at the study site is scored as frequent (40-75% percentage cover), occasional (10-39% percentage cover) or rare (<10% percentage cover). Grey indicates species comprising greater than 1% of the annual diet.

Food item	Overall importance (rank)	Overall mean % by mass	Relative abundance
Kunzea ericoides foliage	1	24.83	Frequent
Muehlenbeckia australis foliage	2	22.64	Frequent
Fuchsia excorticata foliage	3	15.09	Rare
Fuchsia excorticata flowers	4	8.55	Rare
Ulex europaeus flowers	5	6.25	Frequent
Pennantia corymbosa fruit	6	3.97	Occasional
Fuchsia excorticata fruit	7	3.78	Rare
Myrsine australis fruit	8	3.30	Occasional
Melicytus ramiflorus foliage	9	1.52	Frequent
Muehlenbeckia australis fruit	10	1.49	Frequent
Invertebrates	11	1.45	Not assessed
Rubus cissoides foliage	12	1.38	Occasional
Rubus cissoides fruit	13	0.85	Occasional
Ranunculus spp. foliage	14	0.84	Frequent
Schefflera digitata fruit	15	0.73	Frequent
Carpodetus serratus fruit	16	0.73	Rare
Griselinia littoralis fruit	17	0.69	Frequent
Aristotelia serrata foliage	19	0.68	Frequent
Cytisus scoparius flowers	20	0.39	Occasional
Schefflera digitata foliage	21	0.28	Frequent
Pittosporum eugenioides fruit	22	0.21	Occasional
Aristotelia serrata fruit	23	0.19	Frequent
Melicytus ramiflorus fruit	24	0.15	Frequent

Table 3.2: Relative abundance of plant species at the study site, detailing how they were used by possums i.e. which tissue types were consumed ('Y' if consumed). The relative abundance of food species are scored as frequent, occasional or rare.

Food group	Scientific name	Relative abundance	Foliage eaten	Flowers eaten	Fruit eaten
Trees	*Aristotelia serrata*	Frequent	Y		Y
	Griselinia littoralis	Frequent			Y
	Melicytus ramiflorus	Frequent	Y		Y
	Pseudowintera colorata	Frequent			Y
	Schefflera digitata	Frequent	Y		Y
	Myrsine australis	Occasional			Y
	Pennantia corymbosa	Occasional			Y
	Pittosporum eugenioides	Occasional			Y
	Carpodetus serratus	Rare			Y
	Fuchsia excorticata	Rare	Y	Y	Y
Shrubs	*Kunzea ericoides*	Frequent	Y		
	Ulex europaeus	Frequent		Y	
	Cytisus scoparius	Occasional		Y	
Vines	*Muehlenbeckia australis*	Frequent	Y		Y
	Rubus cissoides	Occasional	Y		Y

3.4.2 Influence of season and sex on foraging patterns

Season was a significant predictor of both the % mass (Table 3.3) and the frequency of occurrence (Table 3.4) of all the food types. Figure 3.4 illustrates significant seasonal differences in the dietary composition of possums in terms of how frequently food types are consumed by the population. Figure 3.3 illustrates significant seasonal differences in terms of the mean proportion by mass of the diet a food type comprises (Table 3.3).

However, sex was not a significant predictor of the % mass of any of the broad categories of foods included in possum diet (namely; woody foliage, scrub foliage, vine foliage, flowers, fruits and invertebrates) (Table 3.3). Nor was it significant in predicting the frequency of occurrence of any food type in possum diet (Table 3.4). Figures 3.5 and 3.6 illustrate the lack of significant differences between male and female diets.

For male and female stomachs combined, patterns in frequency of occurrence of food types were broadly similar to those for the proportion of dry mass in the diet (Figures 3.3 and 3.4). However, tree foliage occurred equally frequently in the diet in winter and summer (Figure 3.4), but was consumed in significantly smaller amounts in winter compared to summer (Figure 3.3). In autumn and summer, fruits were consumed equally frequently (Figure 3.4), but they made up a significantly greater proportion of the diet by mass in autumn (Fig. 3.3).

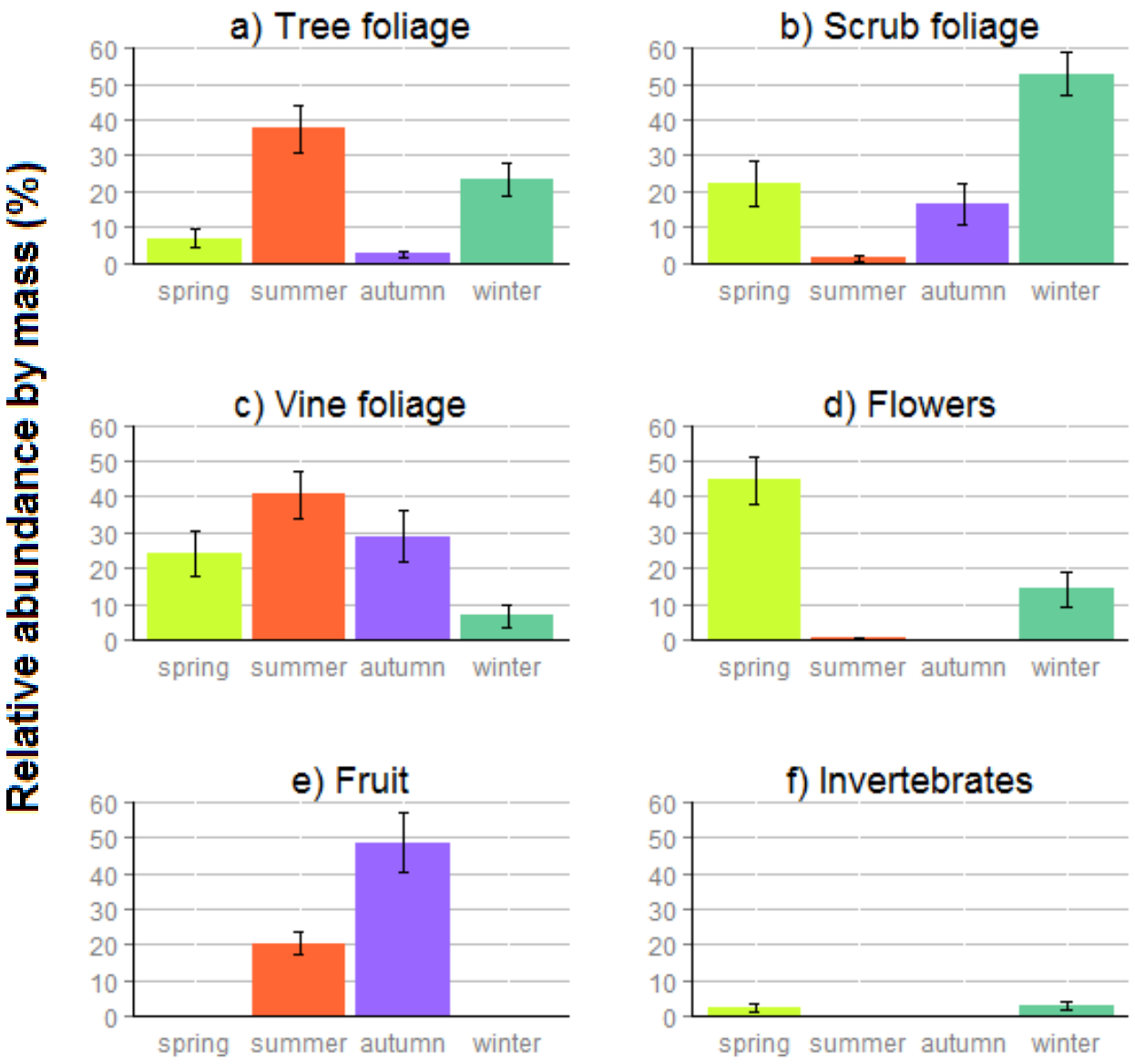

Figure 3.3: Mean percentage composition by dry mass of stomach contents of brushtail possums in each of four seasons for six different categories of food eaten by possums trapped from forest/pasture margins in Otago, New Zealand. Food categories are (a) tree foliage, (b) scrub foliage, (c) vine foliage, (d) flowers, (e) fruit and (f) invertebrates. Error bars show standard errors around the mean.

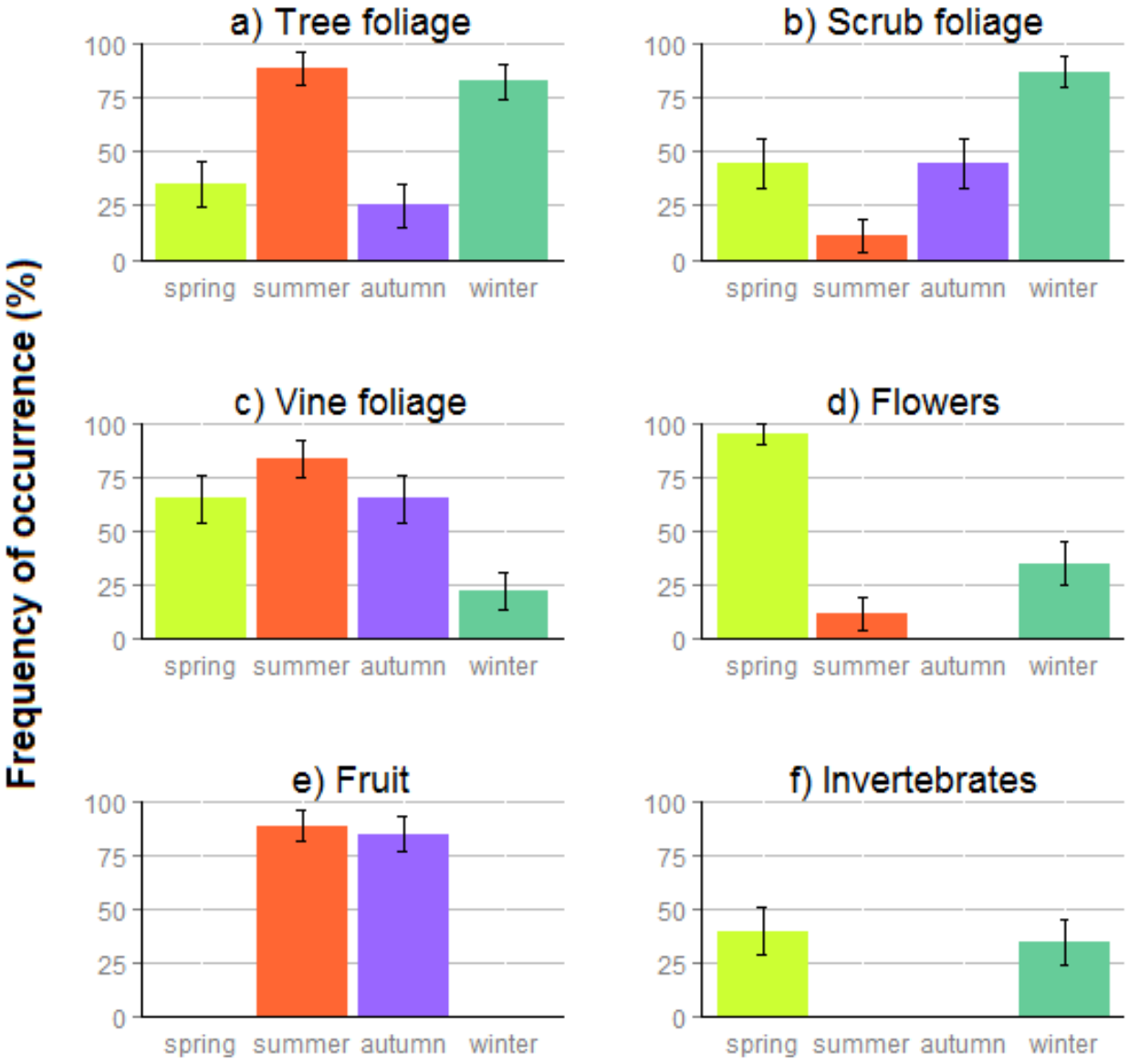

Figure 3.4: Frequency of occurrence of six broad food categories in stomach contents of brushtail possums, in four seasons, at secondary forest-pasture margins in Otago, New Zealand. Food categories are (a) tree foliage, (b) scrub foliage, (c) vine foliage, (d) flowers, (e) fruit and (f) invertebrates. Error bars show standard errors around the mean.

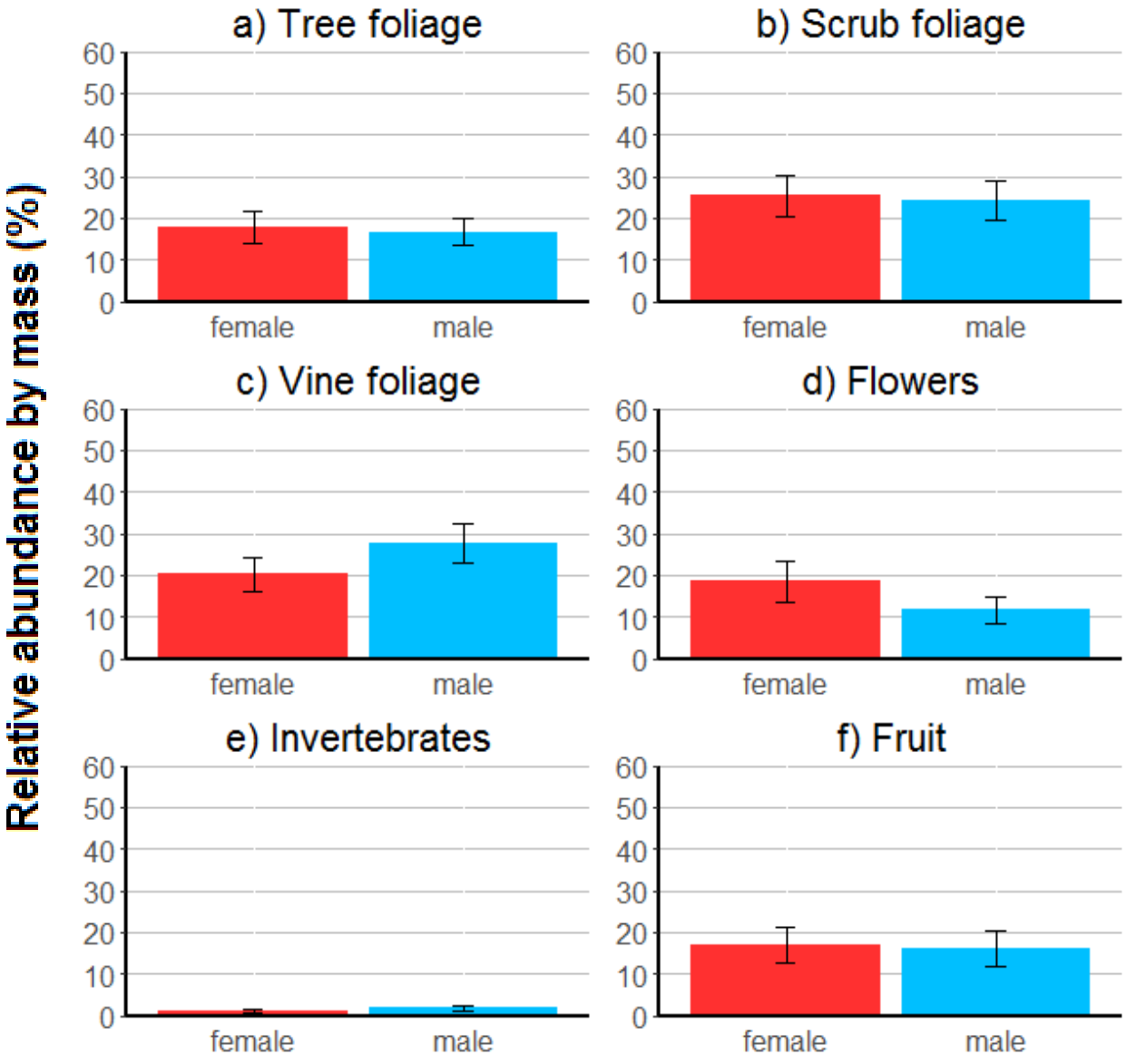

Figure 3.5: Mean percentage composition by dry mass of six broad food categories in the stomach contents of male and female possums trapped from forest/pasture margins in Otago, New Zealand. Food categories are (a) tree foliage, (b) scrub foliage, (c) vine foliage, (d) flowers, (e) fruit and (f) invertebrates. Error bars show standard errors around the mean.

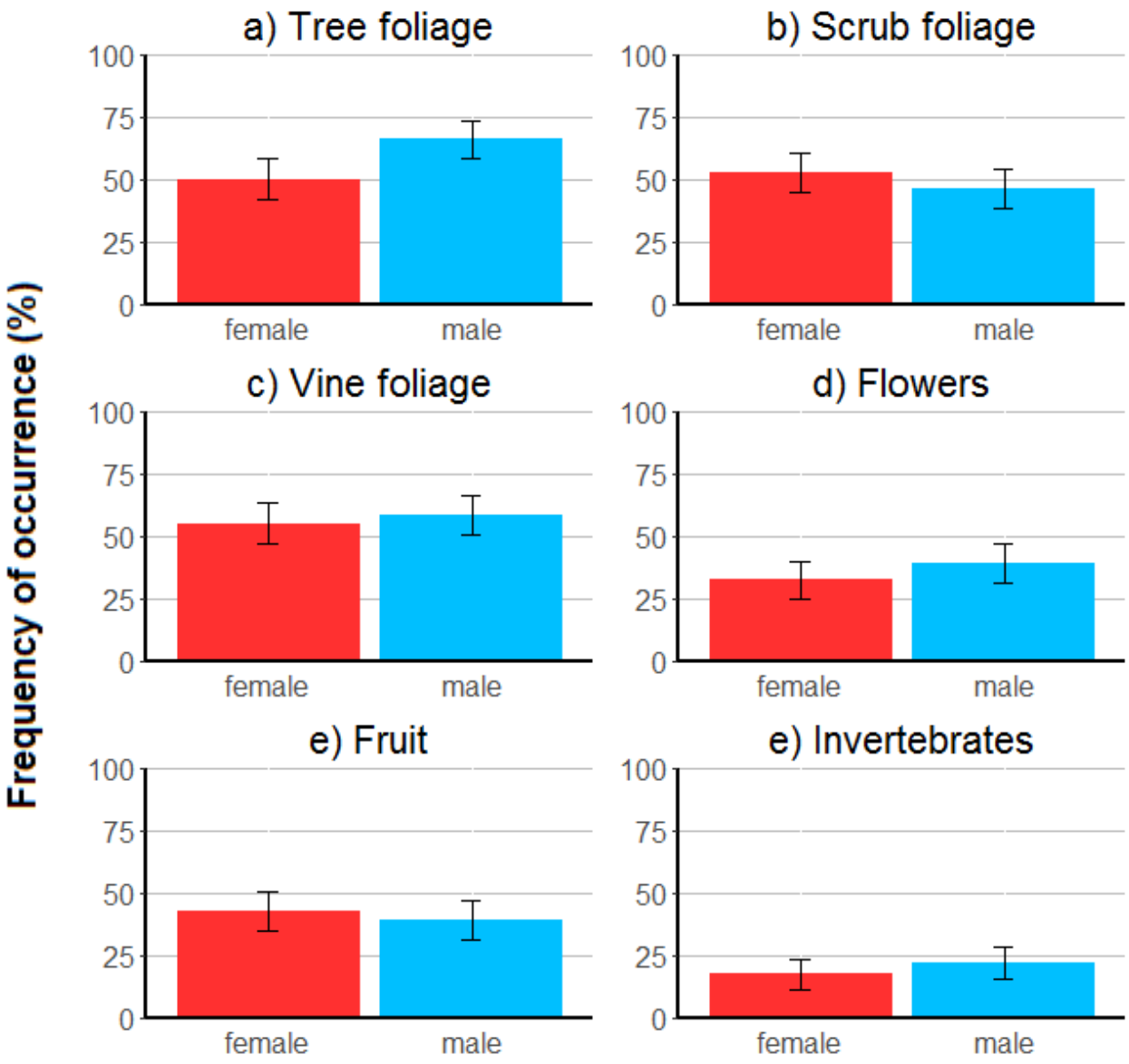

Figure 3.6: Frequency of occurrence of six broad food categories in stomach contents of male and female brushtail possums, at secondary forest-pasture margins in Otago, New Zealand. Food categories are (a) tree foliage, (b) scrub foliage, (c) vine foliage, (d) flowers, (e) fruit and (f) invertebrates. Error bars show standard errors around the mean.

Table 3.3: The effect of season and sex on the relative abundance by mass (M) of the six different food types included in possum diet at forest-pasture margins in Otago, New Zealand. Chi-square (χ^2) and *p*-values for Kruskal-Wallis tests (comparing the proportion of the total diet in each season and the proportion of the total diet for males and females) are given for each food type.

Food group (outcome)	**Predictor**	**Kruskal-wallis Chi-square (χ^2)**	**df**	**p value**
Woody foliage M	Season	31.699	3	.001
	Sex	0.420	1	.517
Scrub foliage M	Season	30.757	3	.001
	Sex	0.075	1	.785
Vine foliage M	Season	18.477	3	.001
	Sex	0.951	1	.329
Flowers M	Season	47.444	3	.001
	Sex	0.001	1	.978
Fruit M	Season	57.916	3	.001
	Sex	0.054	1	.815
Invertebrates M	Season	17.2347	3	.001
	Sex	0.295	1	.587

Table 3.4: The effect of season and sex on the frequency of occurrence (FO) of the 6 different food types that comprised possum diet at forest-pasture margins in Otago, New Zealand. Chi-square (χ^2) and *p*-values for Chi-square tests (comparing the frequency of occurrence in each season and the frequency of occurrence for males and females) are given for each food type.

Food group (outcome)	Predictor	Chi-square (χ^2)	df	p value
Woody foliage FO	Season	26.056	3	.001
	Sex	2.089	1	.148
Scrub foliage FO	Season	23.845	3	.001
	Sex	0.307	1	.579
Vine foliage FO	Season	17.782	3	.001
	Sex	0.103	1	.748
Flowers FO	Season	46.432	3	.001
	Sex	0.375	1	.540
Fruit FO	Season	63.074	3	.001
	Sex	0.101	1	.750
Invertebrates FO	Season	17.804	3	.001
	Sex	0.253	1	.615

3.4.3 Seasonal variation in species composition of diet

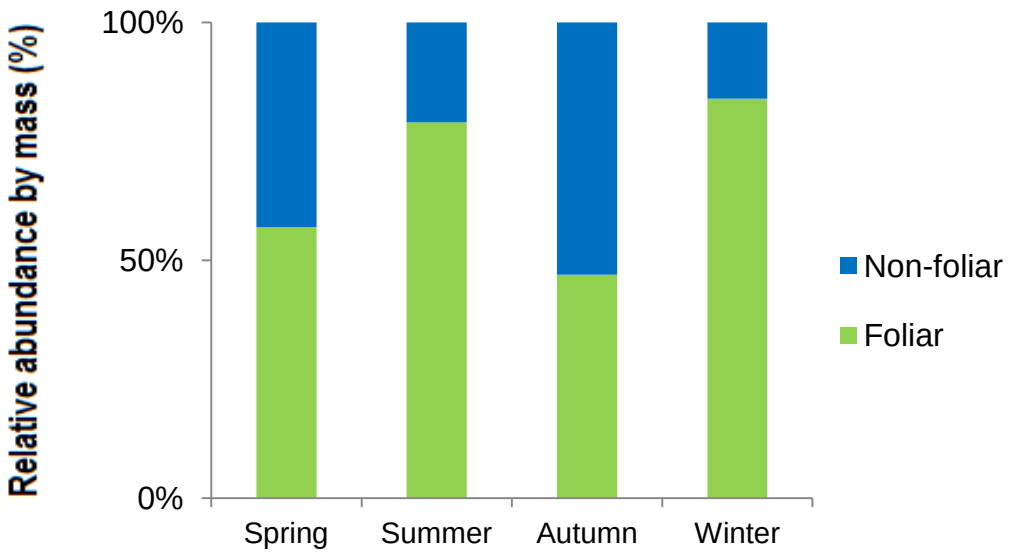

Figure 3.7: The relative proportions of the foliar and non-foliar components of possum diet, in possums stomach contents collected in four seasons at forest-pasture margins in Otago, New Zealand.

Male and female stomachs were combined to further analyse the impact of season on diet composition. Foliage made up the majority of the diet in winter, spring and summer but non-foliar foods were more abundant in the diet in autumn (Figure 3.7). Foliage was particularly dominant in summer and winter, when it comprised 79% and 84% of the dry mass of the diet respectively (Figure 3.7).

Foliage was always a dominant component of annual diet (Table 3.1). The vine *M. australis* dominated in spring and summer, the scrub plant *K. ericoides* dominated diet in winter and spring and *F. excorticata* was most important in winter and in summer (Table 3.5).

The three most dominant dietary components in the annual diet were foliage (of *F. excorticata, M. australis and K. ericoides*), and all three were consumed in all seasons (Table 3.5.), but their frequency and relative abundance in the diet varied. Other foliar foods were limited to supplementary seasonal use. Possums

occasionally consumed relatively small amounts of tree foliage (from *Aristotelia serrata, Shefflera digitata* and *Melicytus ramiflorus*) in some seasons (Table 3.5).

Flowers and fruits were always taken when seasonally available. Gorse (*Ulex europaeus)* flowers and *F. excorticata* flowers were important foods in spring, alongside new-growth *M. australis* leaves (Table 3.5). During winter, flowers remained an important dietary component but utilisation was restricted to *U. europaeus*, because other flowers were not yet available (Table 3.5). In summer, fruit feeding was primarily limited to *F. excorticata* trees, but in autumn the diet included fleshy fruits from a wide range of canopy trees, many of which were used exclusively for their fruits (Table 3.5).

Consumption of larval invertebrates was limited to winter and spring (Table 3.5). Invertebrates were consumed relatively frequently, however their proportion in the diet by dry mass was low (Table 3.5).

Table 3.5: Seasonal consumption of individual food items by possums at the study site, comparing the three different dietary measures (FO, M and PSA). Food items are grouped according to tissue type into foliage, flowers, fruits or other. The greyscale colour scheme is used to highlight important foods; white= 10% and below, light grey = 11-49%, dark grey = 50% and above, of the total M, FO or PSA.

	Foliage							Flowers			Fruit											Other
	FUC EXC	MEL RAM	ARI SER	SHE DIG	KUN ERI	MUE AUS	RUB CIS	ULE EUR	FUC EXC	CYT SCO	FUC EXC	MUE AUS	PEN COR	MYR AUS	RUB CIS	CAR SER	GRI LIT	SHE DIG	MEL RAM	ARI SER	PIT EUG	INVERTE -BRATES
Spring																						
M (%)	6				31	20		14	25	1												3
FO (%)	35				45	65		35	80	10												40
PSA (%)	15				48	32		27	32	11												7
Summer																						
M (%)	32		1	1	1	42	2		1		11	6			4							
FO (%)	83		22	11	11	83	17		11		61	44			17							
PSA (%)	37		7	9	10	50	8		3		19	15			17							
Autumn																						
M (%)	2				15	28	2				6		14	19	1	4	2	2	1	2	2	
FO (%)	25				45	60	10				5		30	25	10	20	10	10	10	5	5	
PSA (%)	9				26	46	17				47		46	55	14	16	21	25	6	55	67	
Winter																						
M (%)	13	3			62	2	4	12														4
FO (%)	65	17			87	9	13	35														26
PSA (%)	18	26			66	49	20	36														9

3.4.4 Graphical approach results

Diet width was narrowest in winter and spring, when seven different items were consumed. Diet width increased in summer to 10 food items and was at its highest in autumn at 14 different items (Table 3.5). The increase in the number of food items in autumn was almost entirely comprised of fruits (Table 3.5).

Figure 3.6 illustrates seasonal differences in possum foraging strategies. Foods are plotted in relation to their % PSA and % FO, following the method Amundsen et al. (1996), and the distribution of points along the axis and diagonals are examined to determine foraging strategies (Figure 3.2).

The Food Importance diagonal (lower left to upper right) varied seasonally (Figure 3.8). 'Important' foods were defined as having a high FO and a high PSA, i.e. were consumed by the majority of individuals, and in relatively large amounts. The importance of different foods varied throughout the year. Most notably; *K. ericoides* dominated in winter (but was also fairly important in all seasons) (Figure 3.8); during spring deciduous leaves of *M. australis* and *F. excorticata* flowers became particularly important (Figure 3.8). Foliage from *F. excorticata* and *M. australis* were the most dominant foods in summer (Figure 3.8). In autumn, *M. australis* foliage was still important, but was consumed alongside a range of fruits, which were consumed in large amounts (Figure 3.8).

The lack of points in the upper right side of the diagrams infers a broad niche width. This pattern was consistent in all seasons. The feeding strategy (assessed by the vertical axis) used by possums was polyphagous in all seasons: a wide range of food items was taken and the majority of points occurred below 50% PSA on the vertical axis (Figure 3.8), meaning each of the food items was eaten only occasionally (as opposed to points in the upper half of the graph which would have been specialised upon either by individual predators (upper left) or by the population (upper right)). However, the feeding strategy of the population in winter did involve a degree of specialisation on *K. ericoides* (as it is located in the upper right of the diagram).

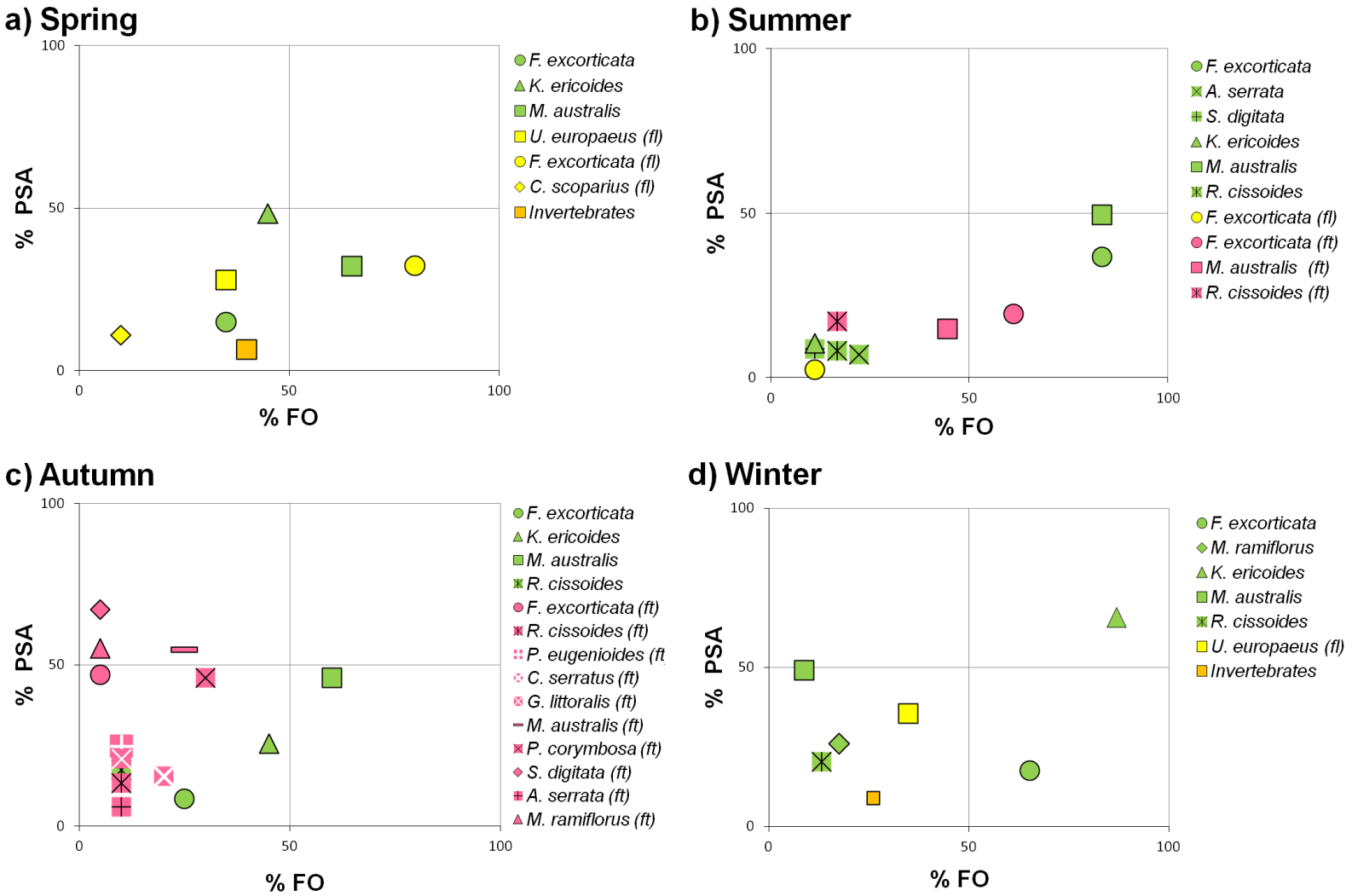

Figure 3.8: Graphical analysis in which foods are plotted in according to their Frequency of Occurrence (% FO) and Prey Specific Abundance (% PSA) in a) spring, b) summer, c) autumn and d) winter diets of brushtail possums at forest pasture margins in New Zealand. Samples are of foliage unless specified as flowers (fl) or fruit (ft).

3.5 Discussion

3.5.1 Possum diet and the availability of foods

Possums were polyphagous in all seasons and consumed a wide variety of food items, including foliage, flowers and fruits from a range of plants as well as invertebrates. Some important plant food items were frequently available in the landscape (e.g*. K. ericoides* and *M. australis*), but other important food items occurred only occasionally or rarely. Most notable was *F. excorticata,* which was rare at the study site, but was heavily used for its foliage, flowers and fruits. Individuals are said to be selective if they utilise resources disproportionately to their availability; the level of this disproportion defines their relative preference for these resources (Johnson 1980). Therefore it seems fair to say that *F. excorticata* is a highly preferred possum food at forest-pasture margins. Other studies have also found *F. excorticata* to be a highly preferred food. Consequently, it has been reduced throughout much of its range by brushtail possums because they can completely defoliate and kill individual trees (Pekelharing et al. 1998; Sweetapple & Nugent 1999).

Possums ate 22 food items, but only three were dominant and together comprised 63 % of the annual diet. This is in keeping with other possums studies. Typically, five or fewer species comprise 50-90 % of possum diet in any one place (Green 1984). Dominant dietary components can be non-foliar, with foliage from seral shrubs and deciduous vines often important. Owen and Norton (1995) recorded 33 food types in possum gut contents, but only four types were dominant and together comprised 68 % of the annual diet by dry mass. These were foliage from the seral shrub *Aristotelia serrata;* foliage from two species of vine, *M. australis* and *Rubus cissoides* and insect larvae.

Findings in this study did not support the original hypothesis (H1). Possum use of foods was not limited to those occurring frequently in the landscape, suggesting that the relative quality or profitability of foods must also be important in determining foraging strategies.

3.5.2 Use of foliar and non-foliar foods

In this study, supplementary, non-foliar foods were eaten in smaller amounts relative to foliage in most seasons but in autumn the consumption of fruits meant that non-foliar foods comprised a greater proportion of the diet than foliage did. This study confirmed that possums have a staple foliar diet, which itself varies with season, but that they frequently consume supplementary, non-foliar foods in all seasons. Supplementary foods were; invertebrates and flowers in winter and spring, flowers and fruits in summer, and fruits in autumn. Results only partially support our hypothesis (H2). Foliage made up the majority of the diet in winter, spring and summer but non-foliar foods comprised the majority of the diet in autumn. Foliage was particularly dominant in summer (79% M) and winter (84% M).

Although the relative availability of plant species across the site is likely to have remained constant during this study, vegetation in New Zealand temperate forest is characterised by large seasonal changes in the availability of foods consumed by possums; in particular, deciduous leaves, flowers, fruits and fungi. The availability of non-foliar foods (e.g. insects or fruit) is hard to measure and previous studies on possum dietary preferences in the wild have only estimated the availability of foliar foods (e.g. Owen & Norton 1995; Nugent et al. 2001). For example, Owen and Norton (1995) used non-destructive foliage cover estimates of all plant species in a forest to quantify availability in order to provide preference indices for possum food items. The authors were unable to quantify the availability of non-foliar foods in a comparable way to foliage, but also concluded that insects and fruit were both seasonally important in possum diet. This study had similar limitations in that it did not quantify the availability of non-plant foods (e.g. insect larvae and flowers). However, our personal observations of the availability of non-foliar items highlighted the strongly seasonal nature of their availability in the landscape. This study used a scoring system to quantify the relative availability of plant species used by possums at the study site from which both foliar and non-foliar resources were taken which still provided insight into the relative abundance of key food items and the effect on diet composition.

This study found that flowers and fruits were important dietary items. When available, they were found to occur frequently in the diet and/or in large amounts

relative to other foods. Many previous studies have not highlighted the importance of non-foliar foods. It could be argued that this is because they used methods that relied upon cuticle analysis (e.g. from scats or stomach contents) so were biased against the detection of non-foliar foods. The use of the layer separation technique in this study enabled the use of foods without cuticles to be reliably estimated. Some other studies have demonstrated heavy use of fruits and flowers by possums: Williams (1982) concluded that the high proportion of available energy in fruits consumed by possums in broadleaf-podocarp explained increased reproductive success when the abundance of fruits in the previous autumn was high.

Flowers and fruits have very different nutrient ratios to those found in foliage. They often contain high concentrations of soluble sugars and lack the fibre that constrains the digestibility of nutrients in leaves (Yarmolinsky et al. 2009). Previous studies have documented clear preferences for sweetness and/or soluble sugar in a range of mammals, from relatively small frugivorous bats to large mammals, such as roe deer and primates (Ito et al. 2017). Williams (1982) compared the nutritional contents of six species of fruit eaten by possums to the leaf-only proportion of the diet. Results showed that by including fruit in the diet possums could increase their rate of intake of digestible nutrients.

Because supplementary, non-foliar foods used by possums are highly nutritious it has been argued that they must play a key role in influencing the density and reproduction of possum populations (Nugent et al. 2000; Nugent et al. 2001; Ramsey et al. 2002; Cowan 2005). Hence, it is important to quantify the use of these items in areas where possums reach high densities and/or are of significant conservation concern.

In this study, all three of the most important foliage food types in possum diet *(F. excorticata, K. ericoides* and *M. australis)* are tree or vines commonly regarded as short-lived seral species in New Zealand forests (Wardle 1991). In this case, out of our three dominant diet species, *F. excorticata* is one of the few fully deciduous trees in New Zealand, *M. australis* is deciduous in the south of New Zealand and *K. ericoides* has relatively short-lived leaves (Wardle, 1991). In spring, new-growth and immature woody plant foliage is associated with higher concentrations of key

nutrients e.g. N and P (Kuhajek et al. 2006) and lower concentrations of digestibility-reducing compounds compared to mature foliage (Marsh et al. 2018). Other diet studies in New Zealand and Australia have shown that seral species are commonly preferred species in brushtail possum diet (e.g. Green 1984; Proctor-Gray 1984). This could be explained by a relationship between increased chemical defence and increased leaf persistence (Opler et al. 1980). Furthermore, relative leaf palatability has been shown to be greatest in early successional (seral) species (Cates & Orians 1975). It has been suggested that increased palatability may relate to the tendency of seral species to invest less in chemical defence in their short-lived leaves (Owen & Norton 1995). This could explain the results found which indicated possum preference for seral and deciduous foliage. The reasons for particularly high possum densities in more floristically diverse broadleaf-podocarp forests have previously been related to higher palatability of foliage in comparison to other forest types, increased presence of seral-woody plant species and a greater diversity of vegetation and alternative food, particularly in the understorey (Nugent et al. 2000).

3.5.3 Influence of sex and season on diet composition

Several polyphagous mammal species have been shown to display sex-specific foraging strategies. Female red kangaroos *(Megalela rufa)* feed selectively on nitrogen-rich forbs (Newsome 1980) to meet the nutritional demands of reproduction and female ship rats (*Rattus rattus*) select high-protein arthropods over plant foods for similar reasons (Gales 1982). The polygynous mating system of possums means males travel much greater distances during the breeding season, which increases their energy use relative to other seasons. Females are solely responsible for parental care; young are weaned in the pouch and then as back-riders for their first 240 days (Green 1984; Cowan 2005). Hence, it could be predicted that the metabolic demands of pregnancy and lactation for adult female possums are likely to exceed the reproductive requirements of adult males (Fletcher & Selwood 2000). However, this study did not find any significant influence of sex on the frequency at which food types were taken or the relative amounts eaten (H3). The similarity in the diet composition of males and females could be explained by their similar body size and mass (Cowan 2005) that may result in similar maintenance requirements. It is also possible that sex differences

may exist but were not captured due to the size of the sample or the choice of seasons in which data were collected.

Season significantly influenced the dietary composition of possums (H3). Both in how frequently food types were taken at the population level and their relative proportion of the diet. Adjusting diet width and species composition to match to spatial and temporal heterogeneity in the environment forms a key tactic in the optimal foraging strategies of polyphagous animals. Previous studies have concluded that long term shifts in composition of possum diets are a response to food scarcity (Nugent et al. 2001). However, short-term dietary switching (between seasons) is common, and is thought to be a response to increased food abundance and quality (van Langevelde & Prins 2008). This study also demonstrates that possums adjust their diets seasonally to consume a nutritionally different mix of food types and to consume different relative amounts of those food types. In this study, the ability of possums to 'switch' their diet seasonally based on food availability is illustrated by the significant increase in the consumption of *F. excorticata* and *M. australis* in spring which was associated with a flush of new shoots and leaves, and the near-absence of *M. australis* foliage from possum stomachs in winter which reflects the deciduous nature of this species.

3.5.4 Seasonal changes in diet width, food importance, feeding strategy and niche breadth.

Amundsen et al.'s (1996) graphical synthesis greatly increases the scope for analysing and understanding foraging strategies and patterns in food resource use in herbivores. Most critically, the method allows stomach contents data from field studies to be used more analytically rather than in a purely descriptive context. This advance can be seen as essential to our ability to evaluate theoretical predictions, particularly in terms of optimal foraging theory (Amundsen et al. 1996). Despite this, the authors conclude that most feeding ecology studies are limited to descriptions of diet composition, without further analysis of the associated foraging strategies or the niche width component to resource use. It is suited to herbivores as well as carnivorous predators, but to date, has primarily been applied to fish (e.g. Corrêa et al. 2012; Borcherding et al. 2013) and marine mammals sampled as bycatch (e.g. Romero et al. 2012; Auttila et al. 2015).

Amundsen's graphical model results confirmed that brushtail possums are polyphagous in all seasons although the extent to which individual species dominated the diet varied between seasons. The diet width was greatest in autumn when a greater proportion of the diet was composed of supplementary non-foliar food items, in particular, fruits. Possums occupied a polyphagous niche but the degree of specialisation on food items and the overall foraging strategy still varied with season. There was a degree of specialisation by the entire population in winter (on *K. ericoides* foliage) when non-foliar foods were least abundant in the diet.

The polyphagous-specialist dichotomy is a central concept in both niche theory and foraging theory (e.g. Shipley et al. 2009; Peers et al. 2012). The term 'generalist' is avoided in this thesis because, strictly, it implies ingesting all potential food items in the proportions in which they are encountered. There is little evidence that any animals exhibit no preferences on where they feed or what they ingest. Indeed, possum preference for foliar foods has been shown to be non-random (Owen & Norton 1995) with some species highly preferred i.e. selection was not explained solely by their availability in the landscape. 'Specialist' (either monophagous or oligophagous) foragers are defined by their narrower food niches as opposed to polyphagous foragers which have broader dietary niches (Bernays & Chapman 1994; Shipley et al. 2009). This study showed that possums are polyphagous with a broad niche breadth. The hypothesis of seasonal variation in foraging strategies (H4) was partially supported. Food choice and food importance varied seasonally but possums displayed a polyphagous feeding strategy with a broad niche width in all seasons.

This work has much applied relevance, in particular because understanding resource use in the habitat type in question is critical for reducing both the impact on native biodiversity and the transmission of bovine tuberculosis (Tb). Possum control in New Zealand currently requires an ongoing annual expenditure by the Crown of $42.7 m and by the private sector of $74.8 m. Direct economic losses to the private sector from possums are estimated to be as high as $60m per year (Parliamentary Commissioner for the Environment 1994). Tb Transmission risk is greatest at the interface between native forests and agricultural pastures where wildlife-livestock contact is most likely to occur. Difficulties in achieving control targets are compounded by the fact that this habitat type seems to support

particularly high densities of possums compared with other habitat types (Montague 2000). Possum density is thought to be the single most important predictor of the degree of impact on other biota and on livestock disease (Efford 2000). Despite this, the drivers of high densities of possums at forest-pasture margins are currently not well understood, although it has been speculated that food resources play a key role (Efford 2000).

This study demonstrates that possums are at risk of causing the local extinction of *Fuchsia excorticata* at the study site. At low densities possums are known to defoliate fuchsia but cause little mortality (Pekelharing et al. 1998). However, at high possum densities increased defoliation results in mortality, and in some cases the local extinction of *F. excorticata* (Rose et al. 1993). This suggests possum control is required to protect this highly preferred food from which possums consume foliage, flowers and fruits. Indeed, fuchsia is already rare at the study site. This study adds to the knowledge of resource use and seasonal patterns in possum foraging strategies, and could aid in improving control strategies.

These results have clear implications for strategies for controlling possums in New Zealand because they provide an opportunity for increased spatial and temporal effort both by trapping and ground level poisoning. Possums specialised on *K. ericoides* foliage in winter, and diet width was at a minimum. Therefore, during winter there is greater potential for concentrating baits or poisons close to *K. ericoides* or in areas where it was dominant which could the efficiency of possum control.

Possum preference for seral, deciduous leaves as well as flowers, fruits and invertebrates suggests strong nutritional drivers of foraging strategies at forest-pasture margins. However, there is a gap in our understanding of how possums forage in relation to the nutritional compositions of foods. It has been speculated that food resources play a key role in supporting high densities of possums in some habitat types in New Zealand. Food selection by herbivorous mammals based on the abundance of valuable nutrients and plant defensive chemicals has been well documented (Jensen et al. 2015; Ito et al. 2017). But for possums the nutritional drivers of foraging strategies are not well understood. This will be explored further in Chapters 4 and 5.

Chapter 4: Seasonal variation in nutritional content of plant foods available to brushtail possums at forest-pasture margins in New Zealand.

4.1 Abstract

The palatability of plant tissues to herbivores reflects a balance between the costs of overcoming plant defences and the benefits of consuming tissues containing profiles of nutrients that match the physiological nutrient requirements of the animal. The nutritional composition of plant species used by possums as food in New Zealand has rarely been quantified, leaving a gap in our knowledge of the nutritional context of possum foraging behaviour. It is often hypothesised that foods preferred by possums have higher nutrient concentrations or are more readily digestible but this has yet to be quantified in most habitat types. At forest-pasture margins possums have been shown to exhibit significant variation in their diet composition and foraging strategies, but the nutritional drivers of these behaviours are currently unknown.

In this chapter I present the results of analysing the nutrient profiles of plants sampled from near to the margins between pastures and native forest and shrub fragments in an agricultural landscape in Otago, New Zealand. Ten potential foods (all found in possum stomach contents) were characterised according to total nitrogen, available nitrogen, solubles (a proxy for readily available energy) and dry matter digestibility (a proxy for total available energy). Measures of nutritional quality varied significantly between plant species. The most important dietary items were not always the highest quality. Possums did not consume higher available nitrogen foods or higher energy foods more frequently or more intensively than lower quality foods. However, *Muehlenbeckia australis* formed the biggest proportion of the diet in both summer and autumn and had the highest content of total and available nitrogen. It is proposed that a range of other nutritional factors must also be driving foraging behaviour at forest-pasture margins.

The nutrient chemistry of important foods in possum diets varied between seasons; the deciduous leaves *Muehlenbeckia australis* and *Fuchsia excorticata* had higher available nitrogen contents and were more digestible in spring. The nutrient content of *Kunzea ericoides* foliage did not vary with season. It is the dominant species in the diet in both winter and spring but had a low level of both nitrogen and the proxies for energy compared to other potential foods. Consumption of *K. ericoides* could be explained not by its available nitrogen or energy content, but by the fact that it is closely related to *Eucalyptus* species found in the native habitat of brushtail possums in Australia.

4.2 Introduction

4.2.1 Determinants of food quality for herbivores

To effectively manage populations of invasive herbivores it is critical to understand multiple aspects of their behavioural ecology, including their foraging strategies in their introduced range. In New Zealand, in has been proposed that foods preferred by possums have higher nutrient concentrations or are more readily digestible (Green 1984). However, the nutritional composition of New Zealand plant species used by possums as food has rarely been quantified or used to explain patterns in possum diet composition and resource use.

Nitrogen is often assumed to be the limiting nutrient for herbivores (Mattson 1980; Robbins 1993; White 1993). Total nitrogen concentrations in foliage have been hypothesised to influence the distribution and abundance of arboreal folivores (e.g. Braithwaite et al. 1984; Cork & Catling 1996). However, tannins and fibre are found in all foliage from woody plants and both have a negative effect on the digestion of nitrogen. As a result, not all of the ingested nitrogen is available for assimilation by consumers so total nitrogen alone may be an inadequate measure of nutritional quality (DeGabriel et al. 2008).

More recently, the concentrations of nitrogen, tannins and fibre have all been considered as key correlates of food quality for browsing herbivores (DeGabriel et al. 2008; Moore et al. 2010). However, until 2008 there was no method for measuring their combined effects. The 'availN' *in vitro* assay (DeGabriel et al. 2008), modified by Wallis et al. (2010), was designed to provide an integrative measure of browse quality for herbivores. The assay measures the effects of tannins and fibre on the digestibility of nitrogen in browse to estimate the proportion of nitrogen available for assimilation (hereafter referred to as 'available nitrogen'). As a result, available nitrogen concentration is considered to be a more appropriate metric for use as a proxy of food quality than total nitrogen (Wallis et al. 2010). Indeed, available nitrogen has been described as the most useful measure of nutritional quality for mammalian folivores currently available (Windley et al. 2016).

4.2.2 Possum-specific background

In New Zealand, it is often hypothesised that possum foraging decisions can be explained by the nutritional quality of foods (Nugent et al. 2000), but this has only been tested rarely (Windley et al. 2016). In Australia, the nutritional quality of tree foliage is often shown to be a significant predictor of preference (e.g. Moore & Foley 2005; Wallis et al. 2012). Furthermore, it has been shown that the available nitrogen concentration of browse predicts reproductive success of possums in *Eucalypt* woodland (DeGabriel et al. 2009). In the first study in a New Zealand indigenous forest, Windley et al. (2016) found that there was significant variation in nutritional quality between tree species and that there was a positive relationship between the available nitrogen concentration of foliage and the severity of browse damage by possums. This verification of the importance of foliar nutritional quality, particularly the concentration of available nitrogen, suggests that it may be important in explaining the foraging behaviour of possums at forest-pasture margins.

We hypothesise that the nutritional composition of different foods is likely to vary significantly, but we don't know the specifics of these relationships, because the chemical composition of NZ foods, especially the non-leaf foods, are so unknown. Critical aspects of possum foraging behaviour can't be understood without characterising some of the non-leaf foods and how their nutritional content may influence foraging behaviour/decisions in possums in NZ. It is particularly important to understand the role of supplementary non-leaf foods (e.g. flowers) when examining the nutritional composition of possum foods.

4.2.3 Aims, objectives and hypotheses

Plant foods contain varying amounts of nutrition for herbivores. The aim of this study is to first characterise foods available to possums at forest-pasture margins and to investigate whether there is significant variation between the foods in terms of their nutritional content.

The nutritional value of possum foods at forest-pasture margins is quantified according to the concentrations of total nitrogen, available nitrogen, solubles and digestible dry matter. 'Solubles' measures the solubility of cell contents in buffered

water and is used as a measure of the soluble cell contents that would be readily digested and absorbed. Total digestibility of dry organic matter (which includes soluble organic matter as well as the proportion of digestible polysaccharides such as cellulose), was used as a proxy total available energy content. This allows the foraging behaviour of brushtail possums at forest-pasture margins to be investigated in relation to the nutritional content of foods.

The association between the relative importance of potential foods in the diet and the nutritional characteristics of potential foods is tested; do possums consume foods more frequently or more intensively when they contain higher concentrations of nutrients and/or are more digestible? Finally, because possum diet and foraging pathway characteristics are known to vary significantly with season, seasonal variation in the nutritional chemistry of the three dominant possum dietary items is investigated. The aim is to determine if seasonal changes in foraging strategies can be explained by changes in the relative quality of foliage.

The following hypotheses are tested:

- **H1:** There will be significant variation between different potential foods in all the nutritional chemistry metrics (total nitrogen, available nitrogen, solubles and dry matter digestibility).
- **H2:** Flowers will be more digestible and contain significantly higher amounts of solubles, but will contain less nitrogen and available nitrogen, compared to leaves.
- **H3:** Foods with higher nutrient contents will comprise higher proportions of the diet and/or occur more frequently than those with lower nutrient contents.
- **H4:** The nutritional chemistry of the three most dominant items in the diet will vary significantly between seasons.

4.3 Methods

4.3.1 Sampling design

Three sets of samples of potential food items were collected for analysis of their nutrient contents (Table 4.1).

1) **Important leaves.** The first set was collected on the basis that they were the most important possum food items (Chapter 3), and was comprised of *Kunzea ericoides* (kanuka) *Muehlenbeckia australis* (muehlenbeckia) and *Fuchsia excorticata* (fuchsia). The contribution of each to the annual diet exceeded 15% and together, these three species accounted for 63 % of the annual diet of possums at forest-pasture margins in New Zealand (Table 3.1) and so were classed as important foods

2) **Unimportant leaves.** The second set comprised samples of three species of leaves that were occasionally consumed by possums, but in much smaller amounts relative to other foods, despite occurring frequently at the study site. Namely, *Aristotelia serrata* (wineberry), *Melicytus ramiflorus* (mähoe), *Schefflera digitata* (päte), and a mixed sample of the sward from pastures available to possums but not found in stomach contents (Chapter 3). In order to be included as 'unimportant' the contribution to the annual diet of these species was less than 2%, but their availability was frequent across the site.

3) **Flowers**. The third set consisted of flowers of three species consumed by possums on a seasonal basis, which were *F. excorticata, Ulex europaeus* (gorse) and *Cytisus scoparius* (broom). During spring, flowers comprised on average 40% of the dry mass of the diet and flowers of two species (*F. excorticata* and *U. europaeus*) were ranked 4th and 5th in the annual diet of possums (Chapter 3).

Table 4.1: Details of the groups of species collected and sample sizes analysed at forest-pasture margins in Otago, New Zealand. Letters denote species abundance across the site as; frequent (F), occasional (O) or rare (R) (Chapter 3).

Group1 – Important leaves	**Group 2 – Unimportant leaves**	**Group 3 – Flowers**
Kunzea ericoides (F) (kanuka) n = 18	*Aristotelia serrata* (F) (wineberry) n = 5	*Fuchsia excorticata* (R) (fuchsia flowers) n= 6
Muehlenbeckia australis (F) (muehlenbeckia) n = 18	*Melicytus ramiflorus* (F) (mähoe) n = 5	*Ulex europaeus* (F) (gorse flowers) n = 11
Fuchsia excorticata (R) (fuchsia) n = 25	*Schefflera digitata* (F) (päte) n = 3	*Cytisus scoparius* (O) (broom flowers) n = 2
	Pasture mix (F) (pasture) n = 6	

4.3.2 Collection and preparation of plant tissues

The locations of plant tissue sampling sites were designed to be immediately adjacent to sites from which animals were trapped for stomach contents analysis (Chapter 3), as described in Chapter 2. Sampling of the dominant dietary items was carried out in all four seasons: August 2012 (winter), October 2012 (spring), January 2013 (summer) and March 2013 (autumn). Tissues from unimportant items in the diet were sampled in winter and summer. Non-foliar foods were collected when present in the landscape which was spring for all three species and also winter for gorse flowers.

For each food item under examination in any one season, three replicate samples from different individual trees or plants were collected. Sample material (approximately 50 g wet weight) was collected from each individual tree or shrub at eight compass point locations in its canopy (N, NE, E, SE, S, SW, W and NW) before pooling the samples into labelled paper bags. Material from vines was sampled from eight different haphazard locations on individual plants and then pooled in the same way.

Plant samples were placed on ice in the field and frozen to -20 ^{0}C within 6 hours of collection. Frozen samples were freeze-dried for at least 48 h at a pressure typically of 15 x 10^{-3} mBar and not exceeding 133 x 10^{-3} mBar. Finally, all samples were ground through the 1-mm screen of a Tecator Cyclotec mill and stored in air-tight containers kept in the dark.

4.3.3 Measures of food chemistry

Four measures of the dried material were quantified: the ‘availN’ assay was used to measure the solubles, dry matter digestibility and available nitrogen content of samples, whilst total N was measured separately using an elemental analyser. All traits are presented as a percentage of dry matter.

4.3.3.1 The availN assay

In vitro pepsin/cellulose digestion simulates digestion in mammalian herbivores, with pepsin simulating protein digestion and a crude cellulase preparation

digesting fibre. This assay was used to determine the dry matter digestibility and digestibility of nitrogen of leaf samples *in vitro*. Values for the digestibility of nitrogen and the nitrogen concentration of the samples were used to determine the amount of nitrogen available to herbivores in foliage. The assay was also used to determine the percentage of dry matter that is readily-soluble (termed 'solubles' as in the modification of the assay (Wallis et al. 2010)). This was calculated from the loss of mass after an initial 24 incubation in 0.05MTris-BASE buffer (25 mL/sample), hence measures the soluble cell contents.

Samples that had been freeze dried and ground to 1 mm were analysed in triplicate. The specific protocol followed DeGabriel et al. (2008) and Wallis et al. (2010):

- Each porous digestion bag (Ankom F57, Ankom Technology, Macedon, New York) was labelled and weighed. 800 mg (± 5 mg) of dried, ground sample was weighed into each bag, which was then double heat-sealed.

- The *in vitro* digestion was started by placing digestion bags into a 0.05 M Tris-BASE buffer, pH 7.1 (Sigma), 25 ml per sample, for 24 h in a moving incubator at 37 ^{0}C.

- After 24 h, samples were removed from the buffer, rinsed in distilled water and then washed thoroughly. Washing involved placing the sample bags into a large flask of distilled water. The flask was covered at the top and inverted twenty times to mix it thoroughly. After this, the washing water was discarded and replaced by clean distilled water. This process was repeated ten times until the samples were thoroughly washed from any remaining solution.

- Sample bags containing residues were dried at 50 ^{0}C for 48 h. The bags were re-weighed when dry. The loss of mass after this initial step is referred to as the solubles. The solubles as a percentage of dry matter is calculated using the dry masses of the sample (g) and the sample residue after 1st step of digestion in buffer (g).

- *In vitro* protein digestion was started by adding digestion bags to a solution containing 2 g 1:10,000 pepsin (Bacto, Liverpool, Australia) in 1 litre 0.1 N HCl (pH 1.0) with 35 ml per digestion bag and then incubated at 37 ^{0}C for 24 h.

- After the 24 h incubation in the pepsin solution, all digestion bags were washed thoroughly as described earlier before starting a cellulase digestion.

- In this step, 35 ml per sample of a solution containing 6.25 g cellulase (Onazuka 3S, Yakult, Japan) in 0.1 M acetate buffer pH 4.75 (made using 6.8 g sodium acetate and 2.9 ml glacial acetic acid per litre) was added to an incubating bucket.

- After 48 h, digestion bags were removed, washed as before and then dried at 50 ^{0}C for 48 h.

- Bags containing undigested residues were then weighed to determine the weight of dry residue which allows calculation of the dry matter digestibility using the initial weight of the undigested sample correcting for bag weight.

- The nitrogen content of the residues for each sample (in the same way as total nitrogen above) was determined to calculate values for this digestible or 'available' nitrogen. Available nitrogen concentration was determined by multiplying the nitrogen digestibility of each sample (%) by the concentration of nitrogen in the original leaf sample (g per 100 g DM).

4.3.3.2 Total Nitrogen determination

Total N was measured separately at Hawkesbury Institute for the Environment, Richmond, Australia using an LECO TruMac Elemental Analyser. The analyzer was calibrated against manufacturer's standards with nitrogen and carbon concentrations that fell within the range expected in the samples.

4.3.4 Statistical analyses

To test H1 (there will be significant variation between food items in all measures of their nutritional chemistry) the mean total nitrogen, available nitrogen, solubles and dry matter digestibility was compared between ten available food items, using 1-way ANOVAs.

To test H2 (flowers will be more digestible and contain significantly higher amounts of solubles, but will contain less nitrogen and available nitrogen, compared to leaves) I look for significant differences in terms of the nutrient metrics between flowers and foliage.

To test H3 (foods that are more nutritionally favourable will be consumed in larger relative amounts and/or more frequently), the relationships between the abundance or frequency of potential food items in the diet and their nutrient concentrations were tested using non-parametric Spearman correlation tests.

To test H4 (there will be significant seasonal variation in the nutritional chemistry of food items), differences between seasons in each of the four nutrient concentrations were tested using 1-way ANOVAs. The significance threshold used in this research was set at $p < 0.05$. All statistical analyses were performed in R 3.2.1.

4.4 Results

4.4.1 Differences between potential food items

There were significant differences in total nitrogen concentration between the ten potential foods ($F_{(9,125)}$ = 35.00, p = .001), available nitrogen concentration ($F_{(9,125)}$ = 41.23, p = .001), solubles ($F_{(9,125)}$ = 47.32, p = .001) and dry matter digestibility ($F_{(9,125)}$ = 70.97, p = .001) (Figure 4.1).

Concentrations of total nitrogen ranged from 1.9 – 3.9 % (Figure 4.1a) and concentrations of available nitrogen ranged from 0.6 – 3.4 % (Figure 4.1b). The highest values of total nitrogen were found in foliage from the deciduous vine *M. australis* and in a mixture of pasture species (Figure 4.1a). There was also significantly more available nitrogen in *M. australis* foliage, pasture and *Cytisus scoparius* (broom*)* flowers than in other species (Figure 4.1b). Available nitrogen was lowest in *K. ericoides* foliage, *F. excorticata* foliage and *F. excorticata* flowers (Figure 4.1b).

Soluble contents ranged from 23 – 47 %. Flowers had significantly higher concentrations of soluble matter than most foliage (Figure 4.1c). The lowest solubles concentrations were found in *F. excorticata* and *K. ericoides* foliage. The dry matter digestibility of foliage ranged from 33 – 75 %, and was significantly lower in *K. ericoides* foliage than in any other species (Figure 4.1d).

Flowers were higher in soluble matter than most leaves, but had comparable levels of digestibility and concentrations of total and available nitrogen to many of the plant species found at the study site (Figure 4.1). There were significant differences in nutrient metrics between the three important leaf species: *M. australis* had significantly higher total nitrogen (Figure 4.1a), available nitrogen (Figure 4.1b) and solubles (Figure 4.1c) concentrations compared to the other two important leaf species (*M. australis* and *K. ericoides*). The dry matter digestibility of *M. australis* and *F. excorticata* were significantly greater than that of *K. ericoides* (Figure 4.1d).

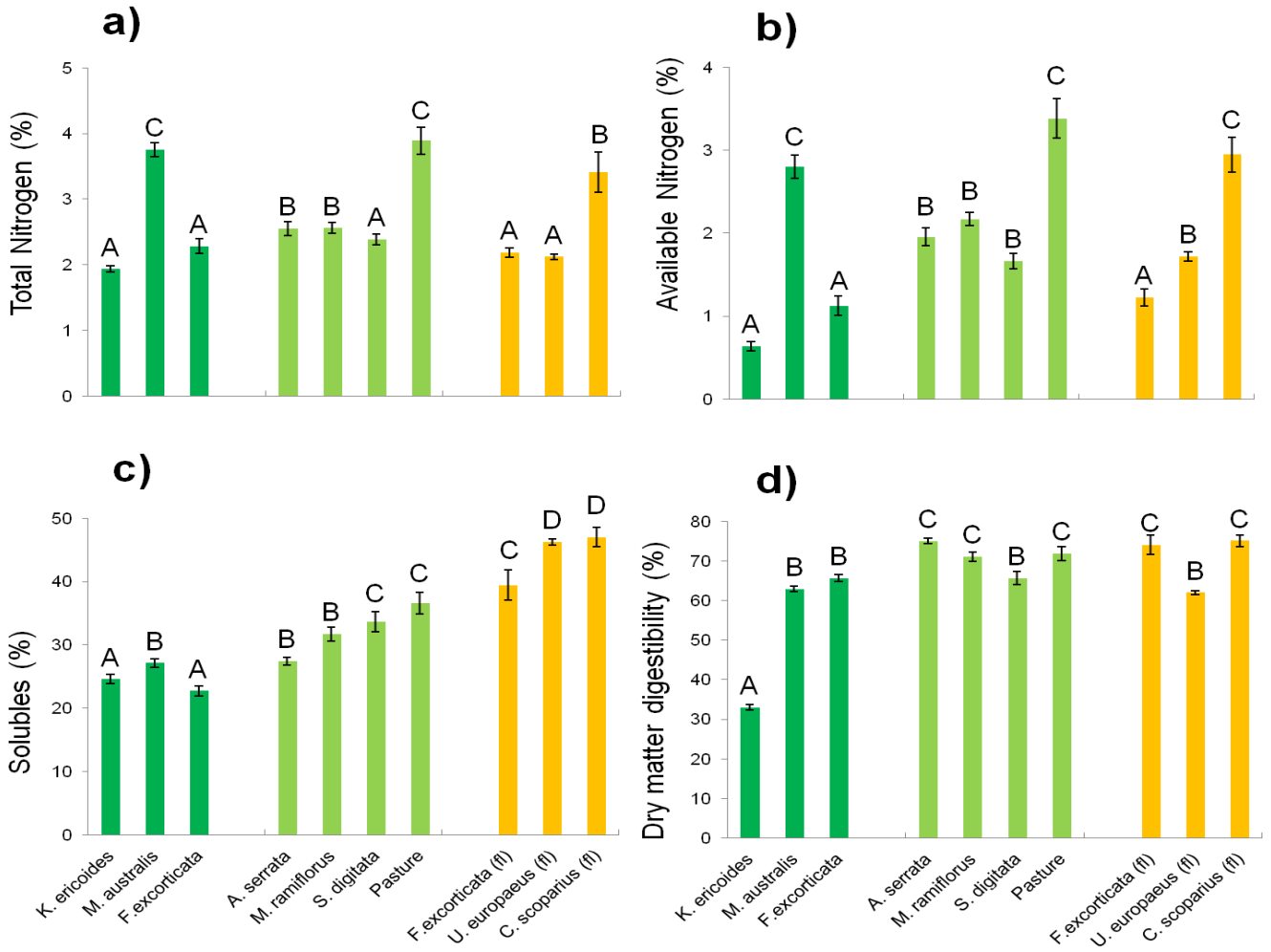

Figure 4.1: The nutritional characteristics of ten potential possum foods, measured according to concentrations of a) total nitrogen, b) available nitrogen, c) solubles and d) dry matter digestibility for important leaves (dark green), unimportant leaves (light green) and flowers (orange) in the diet of brushtail possums at forest-pasture margins in Otago, New Zealand . All items are foliage except where marked (fl) for flowers. Means that do not share the same letter are significantly different at $p < .05$.

4.4.2 Relationship between consumption and food quality

Neither the frequency at which items were consumed, nor their relative abundance in the diet, was significantly associated with any of the measures of nutritional chemistry (total nitrogen, available nitrogen, solubles and dry matter digestibility) (Table 4.2).

Table 4.2: Spearman rank correlation coefficients describing the association between both the frequency of occurrence in the diet (FO) and relative abundance in the diet by mass (M) and the nutrient content of potential foods in terms of total nitrogen, available nitrogen, solubles and dry matter digestibility) at forest-pasture margins in New Zealand.

		r_s	df	p value
Total nitrogen (%)	FO (%)	+ .29	20	.206
	M (%)	+ .12	20	.598
Available nitrogen (%)	FO (%)	+ .19	20	.420
	M (%)	+ .04	20	.856
Solubles (%)	FO (%)	- .23	20	.311
	M (%)	- .22	20	.342
Dry matter digestibility (%)	FO (%)	- .05	20	.831
	M (%)	- .25	20	.271

4.4.3 Seasonal differences in important leaves

The nutrient composition of *K. ericoides* foliage did not vary significantly between seasons in terms of any of the nutrient measures (Figure 4.2). However, there was significant seasonal variation in the nutrient chemistry in both *M. australis* and *F. excorticata* foliage (Figure 4.2).

There were significant seasonal changes in *F. excorticata* foliage in total nitrogen concentration ($F_{(3,21)}$ = 7.341, p = .002), available nitrogen concentration ($F_{(3,21)}$ = 6.866, p = .002) and solubles ($F_{(3,21)}$ = 5.203, p = .008). *M. australis* foliage also varied seasonally in the concentration of solubles ($F_{(2,15)}$ =7.214, p = .006) and the digestibility of dry matter ($F_{(2,15)}$ = 4.224, p = .035) (Figure 4.2).

Tukey post-hoc testing revealed that in winter, *F. excorticata* foliage contained significantly more total nitrogen (Figure 4.2a) and available nitrogen (Figure 4.2b) than it did in summer or in autumn, other comparisons were not significant. In spring, the concentration of solubles in *F. excorticata* foliage was significantly higher than in it was in autumn, with other differences not significant (Figure 4.2c). The concentration of solubles in *M. australis* foliage was significantly higher in spring than in autumn (Figure 4.2c). The dry matter digestibility of *M. australis* also varied with season, and was significantly greater in spring than it was in summer (Figure 4.2d).

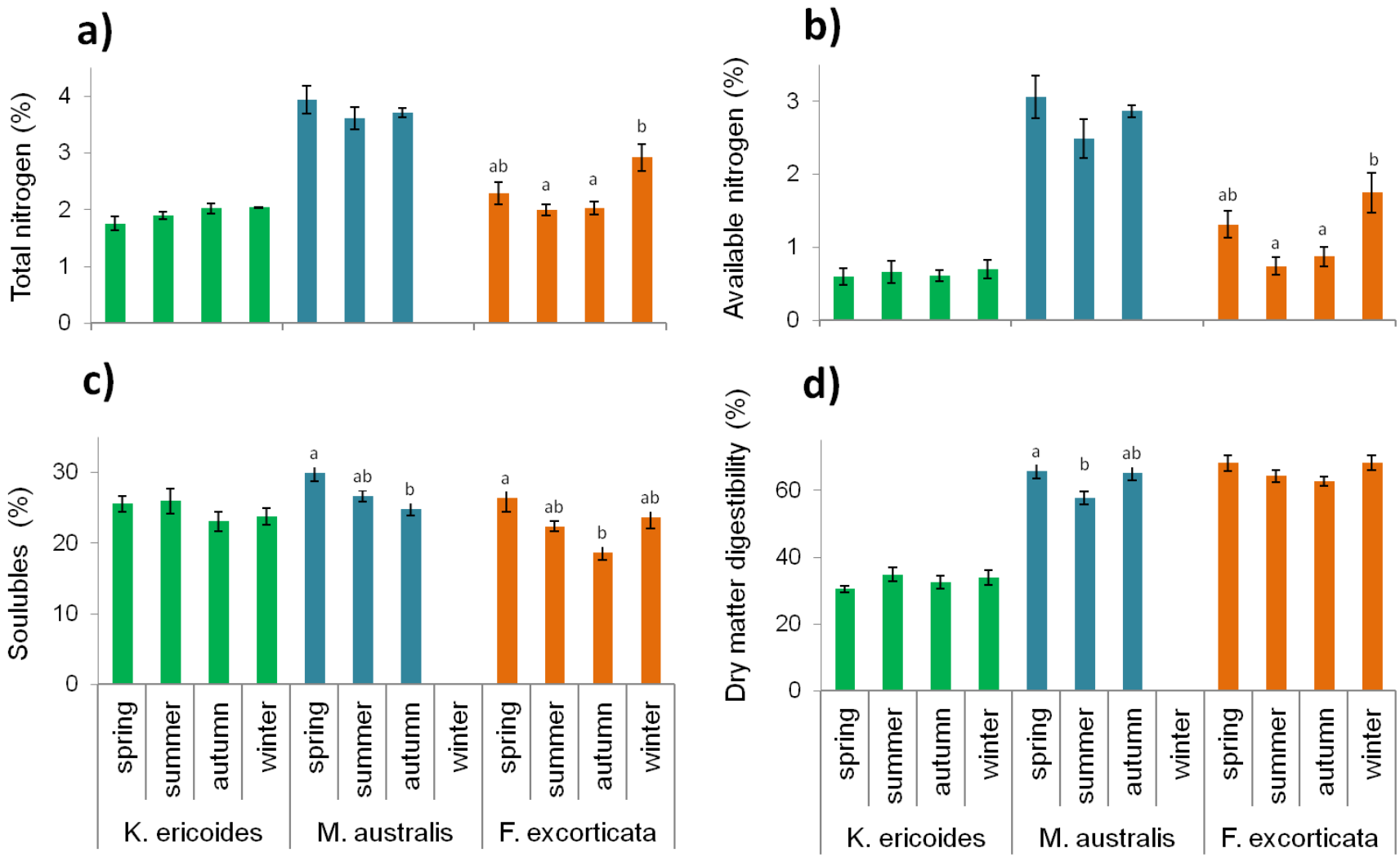

Figure 4.2: Seasonal variation in the food quality measures; a) total nitrogen, b) available nitrogen, c) solubles and d) dry mater digestibility in the three most important dietary species at forest-pasture margins in Otago, New Zealand; *K. ericoides* (green), *M. australis* (blue) and *F. excorticata* foliage (orange). Means that do not share the same letter are significantly different at $p < .05$.

4.5 Discussion

4.5.1 Differences between potential food items

There were significant differences in nutrient content between potential foods available to possums at native forest-pasture margins. The nutrient profiles of ten species (found in stomach contents in Chapter 3), comprising important leaves, unimportant leaves and flowers were characterised. This fills an important gap in our knowledge because foliage of woody plant species in New Zealand has rarely been characterised according to nutrient status, despite a hypothesised link between nutrient contents of potential foods, foraging behaviour of possums and their relative abundance in the environment (Green 1984; Nugent et al. 2000).

Total nitrogen in potential food items ranged from 1.9 – 3.9 % and available nitrogen ranged from 0.6 – 3.4 %. The highest values of total and available nitrogen were found in *M. australis* foliage, pasture and *C. scoparius* (broom) flowers. *M. australis* foliage and *C. scoparius* flowers were eaten but pasture was not detected in stomachs (Chapter 3). Results of this study imply that despite a high nutrient content, pasture plants may be unpalatable to possums for other reasons. For example, physical defences such as silica in grasses may deter feeding. Silica is the primary defence in many grasses (Massey et al. 2007) and has been shown to be important in determining feeding preferences in a range of taxa (e.g. Gali-Muhtasib et al. 1992; Massey & Hartley 2006; Massey et al. 2007). Further work is required to explain why possums do not feed on pasture plants at some forest-pasture margins in New Zealand.

Despite its importance in the diet, *K. ericoides* foliage had the lowest available nitrogen content of all foods sampled. Soluble contents ranged from 23 – 47 % and the digestibility of dry matter in foliage ranged from 33 – 75 % across the ten different potential food items. Important foliage, unimportant foliage and flowers were broadly similar in terms of the range of total and available nitrogen and the digestibility of dry matter contents, but flowers had greater amounts of solubles than foliage did. Few previous studies exist for comparison, but Windley et al. (2016) characterised five native tree species in the North Island of New Zealand and found that foliage contained approximately 1.0 % nitrogen and 0.5 % available

nitrogen, with the exception of *M. ramiflorus* which had consistently higher amounts of total and available nitrogen (approximately 2.0 and 1.5% respectively). In this study, *M. ramiflorus* had values of 2.6 and 2.2 % for total and available nitrogen in. Results therefore suggest that there is variation in traits across the geographic range of species consumed by possums in New Zealand that are likely to be relevant to possum preference and their subsequent impacts.

There were no clear differences between important and unimportant food items in terms of the four measures of nutrient chemistry. In fact, unimportant leaves compared favourably and were often of a higher quality than important leaves. However, unimportant leaves in this study have often been found to be important in other locations and habitats. In silver beech forest, Owen & Norton (1995) found that *A. serrata* was the most dominant component of possum diet, comprising 31 % of the dry mass. In the same study, *M australis* foliage comprised 24 % of the diet but *F. excorticata* only 4 %. *A. serrata* was also the most dominant food (21 %) in a North Island podocarp-hardwood forest (Rogers 1997). *M. ramiflorus* comprised 12 % of the diet in a mixed hardwood-pasture habitat (Coleman et al. 1985), 11% in mähoe remnant forest (O'Cain 1997) and 18 % in a tawa-hardwood forest (Leathwick et al. 1983). Previous studies have measured pasture in possum stomachs. For example, it was estimated that clovers comprised approximately 37 % and grasses 26 % of the stomach contents of possums trapped in pastureland in Canterbury (Gilmore 1967). Previous studies indicate that possum diet composition varies with location and habitat type. However, the results of these diet studies can only be interpreted comparatively with caution, because the methods used to measure diet differed substantially (e.g. faecal analysis compared to stomach contents analysis), particularly in regards to non-foliar items because methods were not as appropriate to detect food without conspicuous cuticles. Furthermore, because diet can vary substantially throughout the year, it may not be appropriate to compare the results of studies that were carried out different seasons.

Pasture foliage was deemed unimportant in this study despite containing comparable nutrient contents to foods that were important in the diet. However, pastures have been shown to be used as a foraging resource in other locations, habitats or at other times of the year. Foliage deemed unimportant in this study

could be more important in other places or at other times of year. One explanation could be that in other locations the different species composition and availability of foods within the landscape resulted in a different nutrient availability context, which then influenced the relative nutritional value of foods available to possums. Therefore, a different set of species may be most favourable. Overall, it can be argued that there will be additional factors (beyond the aspects of nutritional chemistry considered in this study) that may act to influence preferences and foraging behaviour at broadleaf-podocarp forest and pasture margins.

4.5.2 Interspecific differences between important leaves

There was significant variation between the three most dominant dietary items in their nutritional content, which supports the first hypothesis. The highest concentration of total nitrogen, available nitrogen and solubles was found in *M. australis*,. *M. australis* had more than 4 times as much available nitrogen as *K. ericoides* did, as a proportion of the dry matter. The digestibility of the deciduous leaves *M. australis* and *F. excorticata* were similar to each other, but they were two times more digestible than *K. ericoides*. The range in dry matter digestibility was 33 – 75% which was comparable to Windley et al. (2016) who found that the six New Zealand native species they considered ranged between 40 – 65%. In terms of solubles, *M. australis* was again higher than the other two species. Solubles was the only measure in which *K. ericoides* was not significantly lower quality than *F. excorticata.* Solubles were used as a proxy for the most readily available source of energy in the food. Results of this study suggest that the three dominant species in possum diets at forest-pasture margins contain similar amounts of digestible energy, but their nitrogen content varies substantially between species.

K. ericoides was a staple part of possum diet but often ranked lowest in terms of the measures of food quality. In fact, *K ericoides* was never eaten in isolation but was always supplemented with more digestible food or those with higher levels of the nutrients this study measured (e.g. deciduous leaves, flowers, or invertebrates). One explanation may be that *K. ericoides* foliage contains a different key nutrient (or ratio of nutrients) that possums cannot obtain so readily from other foods. The role of other macro- or micro-nutrients in influencing possum diet composition warrants further consideration.

4.5.3 Seasonal differences in important leaves

The second hypothesis was partially supported. There was no significant seasonal variation in any of the nutrient measures of the evergreen foliage sampled from *K. ericoides*, but the deciduous leaves of *M. australis* and *F. excorticata* did vary seasonally. Specifically, *F. excorticata* was variable in terms of both its most readily available source of energy (solubles) and both total nitrogen and available nitrogen content. The nutrient content of *F. excorticata* leaves declined through the growing cycle; nitrogen, available nitrogen and solubles were highest in late winter when the first leaves were forming, but these values were significantly lower in summer and autumn as the leaves aged. Similarly, the digestible energy and solubles content of *M. australis* were greatest in spring which was associated with a flush of new growth.

Possum preference for new growth of deciduous leaves in spring could potentially be explained by increases in the amount of structural material in leaves as they age (Robbins 1993). The results of this study are consistent with this because there was a significant decrease in the digestibility in *M. australis* foliage through the annual growing cycle. Because plant structural material is high in fibre and low in nitrogen, in the absence of plant secondary metabolites (PSMs), a folivores can be predicted to select the youngest leaves (Crawley 1983). In Australia possums have been shown to select younger over older leaves of *Eucalyptus* (Scrivener et al. 2004). However, this is complicated by the finding that younger leaves often have higher concentrations of total phenolics (Cork & Pahl 1984; Hume & Esson 1993; Hume et al. 1996). In *Eucalyptus* forest possums do not always prefer mature leaves with lower PSMs, nor do they always prefer young leaves with higher amounts of available nitrogen (Marsh et al. 2018). Instead, it is the concentration of secondary metabolites in young leaves that determines preference: possums prefer individual trees that produce young leaves with low PSM concentrations (Marsh et al. 2018). Further empirical studies into the determinants of palatability and preference within possum diets in New Zealand could be examined further in experiments with captive animals, as used in Chapter 5.

In New Zealand, Windley et al. (2016) also detected seasonal changes in the quality of foliage consumed by possums: available nitrogen contents and

digestibility of *Weinmannia racemosa* (kamahi) were highest in spring and lowest in autumn. Total nitrogen followed the opposite pattern, being highest in autumn. This was explained by higher tannin contents in autumn which meant that despite higher total nitrogen, available nitrogen was lowest in autumn. The authors concluded that their results supported the role of nutrients in determining the relative palatability of foods which in turn is likely to produce the observed seasonal trends in possum dietary preferences in New Zealand (Windley et al. 2016).

4.5.4 Relationship between diet composition and nutrient content of foods

Variation in the consumption of food items was not well explained by the four measures of nutritional content in this study. Foods with higher nutrient contents did not constitute a significant higher proportion of the diet by frequency or mass therefore the final hypothesis is not supported.

Food selection by herbivores is the result of balancing trade-offs between multiple nutrient and plant defences. These include other quantitative chemical defences (in addition to the digestibility reducing ones evaluated in the availN assay), qualitative defences and a large array of physical defences, all acting as deterrents. This demonstrates the complexity of food selection by herbivores and suggests that explaining the food intake of possums more fully requires further investigation into plant chemistry that was beyond the scope of this study. In particular for plants in New Zealand it would be helpful for future work to explore the role of plant secondary metabolites in determining the palatability of plant tissues to possums.

Nitrogen is not only required to meet the demands of protein synthesis for growth and reproduction but is also used in the detoxification of ingested plant secondary metabolites (Au et al. 2013). Previous studies showed that mammalian herbivores can eat greater concentrations of plant secondary metabolites when consuming high-protein diets; for example, sheep (Villalba & Provenza 2005) and possums (Nersesian et al. 2012). In Australia possums feed extensively on the dominant genus *Eycalyptus* which is known to contain high levels of a variety of plant secondary compounds (Tyndale-Biscoe 2005), A considerable body of research

from Australia has established that several plant secondary metabolites reduce browsing by herbivores and act as feeding deterrents to possums (Iason et al. 2012), e.g. tannins (Marsh et al. 2003; O'Reilly-Wapstra et al. 2005); terpenes (Wiggins et al. 2003); and formylated phloroglucinol compounds (Wallis et al 2002; O'Reilly-Wapstra et al. 2004). In native *Eucalyptus* forest it has been shown that spatial variation in the concentrations of plant secondary metabolites results in patchiness in preferred feeding habitats for possums; this spatial heterogeneity in plant chemistry influences their feeding behaviour (Iason et al. 2012). Specifically, Wiggins et al. (2006) found that possums must switch their diet (change food types while feeding) in order to maximise their intake and therefore their foraging efficiency, because mixed diets are less effected by high concentrations of any one particular plant secondary metabolite.

The current study used pooled samples from individual trees to produce mean nutrient values for each species. However, other studies have found significant spatial and temporal variation in nutritional quality existed both between tree species and between individual trees within a species (e.g. Moore et al. 2010; Windley et al. 2016). As well as nutritional traits, there is also a high degree of intraspecific variation in plant secondary metabolites (Bustos-Segura et al. 2017). Furthermore, Payton (1988) investigated *Metrosideros robusta* (northern rātā) tree use by possums in New Zealand and found that tree age was a significant predictor of the severity of browse damage and defoliation, suggesting another dimension to intraspecific variation. The presence of, and interspecific variation in, other plant secondary metabolites could explain the lack of browsing on some individual trees (Tyndale-Biscoe 2005). Therefore the role of variation between trees in driving foraging studies may also have influenced feeding by possums in this study area but investigating this resolution of spatial variation was beyond the scope of the present project.

The role of plant secondary metabolites in determining palatability of foliage has been investigated elsewhere in New Zealand. Studies of the diet of the Orongorongo valley possum population have revealed that some plant species there are never browsed by possums. These species were found to contain up to 12 % by weight of terpenes (Tyndale-Biscoe 2005), compared to less than 2 % for the most preferred species (Brockie 1992). In Orongorongo, possum browse

damage to *M. robusta* was found to be highly variable between individual trees (Meads 1976). In particular, some trees were very heavily browsed possibly due to an increase in palatability of new growth which is stimulated by browsing (Kuhajek et al. 2006). The presence of, and interspecific variation in, PSMs could also explain the lack of browsing on some individual trees. Kuhajek et al. (2006) found that when trees were totally defoliated the new growth had higher nitrogen, phosphorus and potassium concentrations and reduced tannin content. Significant variation was also found between leaves growing in full sun or shade on the same tree. What was made clear by the present study was that possums actively select their food plants, and plant parts, as opposed to relying solely on the most available species (Tyndale-Biscoe 2005), but the exact mechanisms that explains the patterns in selection is still to be elucidated.

In this study, possum preference for *K. ericoides* foliage is not well explained by the nutrient content results. However, previous studies have found similar results: *Metrosideros* (rata) species make up a large proportion of the diet in many possum studies (Nugent et al. 2000), but have a low nutritional value and high plant secondary metabolite content (e.g. total phenolics). Total nitrogen concentrations of northern and southern rata are less than 1 % of the dry weight (Tyndale-Biscoe 2005). The evolutionary history of possums in Australia could help to explain the potentially counter-intuitive patterns of diet selection in New Zealand: the nitrogen content of *Metrosideros* species is at the lower end of range that is normal for *Eucalyptus* species in Australia (Lambert et al. 1983; Braithwaite et al. 1984), but the higher end of the normal range for potassium and phosphorus (Braithwaite et al. 1984; Cork 1992). Total phenolics in rata foliage were also found to be within the range of those measured in *Eucalyptus* forests in Australia (Cork 1992). However, the *Eucalyptus* forests in Australia studied by Cork (1992) did not support high densities of possums, unlike the forests in New Zealand, despite the similarities in nutrient profiles of the dominant canopy species. This suggests that consumption of non-foliar supplementary foods (e.g. invertebrates (Cowan & Moeed 1987) and fruit (Williams 1982)), and more palatable understory species in New Zealand, may be very important in producing and supporting high density possum populations found there (Tyndale-Biscoe 2005).

The nutrient contents of even the least nutritious species in New Zealand (e.g. *K. ericoides*) are within the range of *Eucalyptus* species in Australia, to which possums have adapted through many years of co-evolution. It is interesting to note that *K. ericoides* and the species of rātā found in New Zealand (*Metrosideros* genus), which are known to be favoured by possums despite their relatively low nutrient content and high plant secondary metabolite content, are in the same family as *Eucalyptus* (Myrtaceae). A possible topic for future consideration could be to investigate whether the shared evolutionary history of possums and *Eucalyptus* in Australia, has resulted in them being adapted to the particular balance in the trade-off between plant defences and nutrient contents for New Zealand representatives of the same family.

Chapter 5: Diet composition and nutrient intake rates of brushtail possums fed mixed diets.

5.1 Abstract

Possum diet composition and dietary preferences have been measured across a range of habitats in New Zealand. However, the nutritional drivers of foraging behaviour in New Zealand are still not well understood. In particular, access to supplementary food has been implicated in maintaining high possum densities that impact native forests, and use of pasture by possums poses a Tb risk to farmers. Despite this, little empirical evidence exists to explain how the nutritional content of foods available to possums at native forest-pasture margins may explain aspects of their foraging behaviour.

I measured changes in the species composition of the diet and the subsequent nutrient intake rates of captive possums in response to the inclusion of supplementary foods (flowers and pasture species) to a basal leaf diet known to be consumed by possums at forest-pasture margins (*Muehlenbeckia australis, Fuchsia excorticata* and *Aristotelia serrata*). Thee diets were tested: leaf only, leaf + flowers and a leaf + pasture. The available nitrogen and digestible energy contents of foods were measured, as was the consumption of each food separately. The nutrient intake rates of possums were calculated for each of the three diets. The intake ratio of available nitrogen to digestible energy was calculated for each of the three diets and assumed to be an intake target ratio.

The relative proportions of the three foliar foods in the basal varied significantly when both pasture and flowers were added to the diet. The intake rates for the total dry matter, available nitrogen and digestible dry matter also varied significantly between the diet treatments: supplementing with flowers reduced the intake rates of dry matter and of nutrients. Supplementing with pasture didn't affect the intake rates of dry matter or digestible energy, but did increase the available nitrogen intake rate.

On all three diets possums maintained a similar intake ratio of available energy to digestible energy. If this is assumed to be a target intake, this target ratio could not have been achieved by consuming any one of the available foods in isolation, suggesting that possums must mix their diets in order to balance multiple nutrient needs.

Results are discussed in relation to three major foraging theories; optimal foraging, nitrogen limitation and Geometric Framework theory. It was concluded that results for possums in captivity support the Geometric Framework theory of nutrition that hypothesises that herbivores eating mixed diets can regulate multiple nutrient requirements simultaneously.

5.2 Introduction

5.2.1 Theoretical background

Optimal foraging theory hypothesises that foraging animals make decisions that maximize some currency of fitness, normally assumed to be the net rate of energy gain, subject to constraints (Stephens & Krebs 1987). However, herbivores were neglected in the development of classic optimal foraging theory and subsequent theory has stressed the role of nitrogen in influencing foraging behaviour. In particular, White's 'Inadequate Environment' hypothesis (1993) argues that nitrogen, not energy, is the most limited currency in determining the growth and reproduction of animals. White (1993) reasoned that because nitrogen is relatively dilute in plant foods, it must be critically important both in limiting herbivore populations and in determining their foraging strategies.

More recently, Simpson and Raubenheimer (1993) have developed their 'Geometric Framework' theory; it states that herbivore foraging traits have evolved to balance multiple nutrient intake requirements. Therefore, animals will select foods in order to simultaneously regulate multiple needs rather than maximise uptake of any one particular nutrient, reaching an optimal intake target ratio of one nutrient to another (Simpson et al. 2004; Raubenheimer et al. 2009; Simpson and Raubenheimer 2012). Use of the Geometric Framework to analyse the intake rate of nutrients has shown that some species previously classified as 'energy-maximisers' do not in fact conform to this assumption in the wild (DeGabriel et al. 2014). For example, black spider monkeys forage to meet daily nitrogen targets, not to maximise daily energy intake, regardless of season, relative food availability or plant secondary metabolites (Felton et al. 2009). This study uses the Geometric Framework theory to explain aspects of possum foraging behaviour.

5.2.2 Possum-specific background

Previous research has measured possum diet composition and diet preferences across a range of habitats in Australia and New Zealand. However, the nutritional motivation for diet choices and foraging behaviour in New Zealand is still not well understood. An increased understanding of the nutritional drivers of possum

foraging behaviour at forest-pasture margins could combine with other findings in this thesis to both increase our understanding of possum impacts and increase the effectiveness of control operations. Furthermore, understanding if, and how, animals benefit from feeding in pastures could help to predict pasture use by possums. This would also mean that the Tb transmission risk posed by possums could be more accurately quantified and reduced.

Previous chapters, and other studies, have established that possums regularly supplement their leaf diet with non-foliar foods such as insects, flowers, fruits etc. Access to supplementary, non-foliar foods has been implicated in producing the high densities of possums found at forest-pasture margins and in other habitat types in New Zealand (Cowan 2001). Chapter 4 demonstrated that flowers contain higher concentrations of energy and similar concentrations of protein compared to commonly consumed leaves. Pasture has also been recorded in possum diets in some locations, but not others. Pasture food (mixtures of herbs and grasses) offers favourable concentrations of nitrogen and energy, despite not being consumed in our study (Chapter 4). This chapter aims to explain some of these patterns by creating a feeding trail which presented three diets to possums in captivity: a basal leaf diet; leaf + flowers; and leaf + pasture.

5.2.3 Aims, objectives and hypotheses

This study aims to understand how the nutritional content of the diet may influence possum foraging behaviour. Specifically, changes to the leaf diet composition and nutrient intake rate in response to the inclusion of supplementary foods in the diet.

Possums housed in captivity will be fed mixed diets, on which the consumption of each food species will be monitored individually. This allows us to investigate how the diet composition (i.e. the relative proportions of food species) varies with treatment diet. When this is combined with nutritional analysis of the food items this then allows for the calculation of the consumption rate of two key nutrients likely to correlate with possum fitness; available nitrogen (as a proxy for protein) and digestible organic matter (as a proxy for energy). Three diets will be compared to investigate the effect of diet supplementing on the overall consumption of dry

matter, as well as the ingestion rates of the two key nutrient measures; energy and protein.

Available nitrogen was chosen as a measure because it is likely to correlate well with the nitrogen available for assimilation by the consumer, and therefore be a closer correlate for fitness than total nitrogen. Total nitrogen is an inferior measure because it doesn't account for the negative effect of fibre and tannins on nitrogen digestibility (DeGabriel et al. 2008). The amount of digestible organic matter in plant material was chosen as a proxy for energy content of the foods consumed.

Finally, the ratio of energy to protein will be calculated for each food type and for each diet, in order to establish a nutritional target intake ratio for these key nutrients.

The following hypotheses are tested:

H1: The relative proportions of different foods consumed will vary with diet.

H2: The total dry mass consumed will vary with diet.

H3: The intake rate of nutrients (i.e. digestible dry matter and available nitrogen) will vary with diet.

5.3 Methods

5.3.1 Source of animals

Animals were wild-caught in standard baited, swing-door cage traps in native forest near Dunedin in January 2014, using the protocol established by the van Heezik research group (Chapter 3). All trapping sites were used, with permission and support from the landowners, for a range of activities under ethics protocol 65/12. All areas were Tb free and not subject to possum control measures during the study.

When an animal was caught, it was removed from the trap into a hessian sack to enable safe handling. Gender was then determined. Juvenile (weighing less than 2.5 kg) and female possums were released immediately at the site they were captured. Adult male possums were anesthetised with an intramuscular injection of Ketamine (100 mg ml^{-1}) and Medetomidine hydrochloride (1 mg ml^{-1}) and health checked by Dr McLeod in the field. By using only male possums I removed a possible cause of variation between individuals. After health checks, possums were ear tagged for identification and anaesthesia was reversed using Atipamazole (5 mg ml^{-1}). Animals were allowed to recover for 30 minutes ensuring airways were clear before transportation to the Hercus Taeri Research Unit animal facility in Dunedin.

5.3.2 Animal housing

Animals were kept in outdoor enclosures with access to cover in group housing conditions for 6 days at the Hercus Taeri Research Unit before the feeding trial began. Hanging sacks and nest boxes were available as well as branches and logs for enrichment. The protocols used at the AgResearch possum facility were adopted in group housing (McLeod et al. 1997).

During the initial days in group housing, animals were weighed and checked daily. Possums were given *ad libitum* access to food and water in group housing. Possums were offered a mixed diet of native tree leaves, apples and possum pellets. Pellets (e.g. rabbit pellets) are used regularly to feed brushtail possums in

captivity (Brown et al. 1996) and they were used previously as a dietary supplement in a pilot study.

After acclimatization to captivity in group housing, the eight possums were transferred to the University of Otago Zoology Department animal facility and housed individually. Individual housing allowed for the measurement of food intake by each individual.

5.3.3 Experimental conditions

The animal housing was kept at an ambient temperature and humidity, and was kept under the same light regime encountered in the wild. Animals were housed individually for the duration of the feeding trial in standard individual, wire metabolism cages (e.g. Foley & Hume 1987a; Foley & Hume 1987b). The cages were suspended on a frame of racks so that the possums were in close proximity (~15 cm) to one another, which minimized their isolation.

Each metabolism cage (0.6 m x 0.6 m x 0.4 m) contained a wooden nest box (28 x 25 x 30 cm) with a solid floor that was used by the possums for sleeping. Water was available *ad libitum*. The status of animals was checked twice daily and they were re-weighed after each 5 day treatment block.

All animals were euthanised at the end of the experiment by an overdose of barbiturate administered by an intra cardiac injection. Animals were sedated prior to euthanasia using Ketamine (100 mg ml^{-1}) and Medetomidine hydrochloride (1 mg ml^{-1}) administered by an intra muscular injection.

5.3.4 The feeding trial

The feeding trial took place in January and February of 2014. The experiment comprised three treatments i.e. three different diets; each treatment lasted 5 days (Table 4.1). Between each treatment was a ‘wash-out’ period of two days during which time possums were fed the same diet as in the subsequent treatment but intake was not included in calculations. This avoided measuring nutrient gain associated with feeding outside the experimental period.

Table 5.1: Experimental design, describing the food species offered to possums during the feeding trial, during three treatments (diets) of 5 days duration each.

Foods offered	Treatment 1 – **Leaf** 5 days	Treatment 2 – **Leaf + flowers** 5 days	Treatment 3 – **Leaf + pasture** 5 days
Basal leaf diet *ad libitum*	*Fuchsia excorticata* *Muehlenbeckia australis* *Aristotelia serrata*	*Fuchsia excorticata* *Muehlenbeckia australis* *Aristotelia serrata*	*Fuchsia excorticata* *Muehlenbeckia australis* *Aristotelia serrata*
Supplement 75 - 100 grams		Flowers from *Rosa spp.*	Pasture – a sample of a mixed grass/herb sward (detailed in section 5.2.5)

5.3.5 Rationale for the species composition of diet treatments

The dietary species chosen for inclusion in the treatments were informed by gut contents analysis of possums caught in equivalent areas to the possums in this study i.e. forest-pasture margins. The leaf diet was comprised of species known to be consumed by possums during summer and was chosen to ensure sufficient nutrition (Foley & Hume 1987b). A pilot study suggested these species would all be eaten in captivity. Chosen leaf species also occurred frequently in the landscape surrounding Dunedin from which plant material was harvested for the feeding trial.

The supplementary foods (pasture and flowers) have been known to be consumed by possums in New Zealand (e.g. Clout 1977; Warburton 1978; Coleman et al. 1985; Cowan 1990a), but pasture species were not found in the gut contents of possums in Chapter 3. Chapter 3 does demonstrate that flowers from native and introduced plants are important dietary components for possums when available in the environment.

Possums are known anecdotally to feed on introduced *Rosa* species in rural and urban gardens in Otago (Bernie McLeod, *pers. comm.*). During this study it was impractical to harvest the necessary amount of flowers from native trees that were flowering at that time (summer) e.g. *Melicytus ramiflorus* (mähoe) or *F. excorticata*. Native flowers were not chosen, mostly due to their very small size making them difficult and timely to handle and prepare. *U. europaeus* would have been suitable but was not flowering during the study period. It was not deemed to be acceptable to remove large quantities of flowering native plants from parks or reserves around Dunedin. In contrast, *Rosa* flowers were relatively large and not difficult to harvest and prepare. Furthermore, roses were plentiful in a local amenity park from which a supply was guaranteed for the duration of the feeding trial.

'Pasture' as a food type was taken as a representative sample of a mixed grass and herbaceous sward on improved pasture typical of that found in lowland farmland in Otago. Hence was comprised of a mix of species likely to be encountered by possums feeding in the wild. Dominant species were the grasses *Agrostis capillaris* (browntop), *Anthoxanthum odoratum* (sweet vernal), *Lolium perenne* (ryegrass), *Dactylis glomerata* (cocksfoot) and *Holcus lanatus* (Yorkshire fog), as well as clovers (*Trifolium* spp.), daisy flatweeds (e.g. dandelion, catsear, hawkbit and daisies), and other herbs.

5.3.6 Consumption data collection

Possums were offered a known amount of freshly-cut plant material daily which initially was approximately 800 g (including stems). This was a larger amount than was consumed in previous studies (Tyndall-Biscoe 2005) and in the pilot study (20 % greater than observed maximum). Of the 800g of leaf material offered,

approximately 500 g was *M. australis*, 150 g was *F. excorticata* and 150 g was *A. serrata*. *M. australis* had relatively high water content and high proportion of stem to leaf therefore its plant material was offered in a greater mass relative to the other two species. During the supplemented diet treatments possums were offered either 75g of flowers or 100g of pasture mix in additional to the basal leaf diet in the same quantities.

The collection and weighing of plant material not consumed by each possum was carried out during the day when possums were sleeping in nest boxes and so minimised disturbance to the animals. The weight of food ingested per night was calculated from the known amounts offered and then remaining for each individual possum, after each feeding night. Control offerings were also used to correct for water loss that would result in a loss of mass not associated with consumption, and data were corrected to reflect this.

5.3.7 Nutritional analyses

Sub-samples of the wet plant material were collected for subsequent nutritional analysis. Samples of each food species were dried and these data were used to calculate the percentage dry matter of each food type. Consumption data were used to calculate the amount in dry grams of each food species consumed per night per individual. Chapter 4 describes the methods used for the analysis and calculation of available nitrogen and digestible organic matter.

5.3.8 Statistical analyses and hypothesis testing

Hypothesis 1 was tested by determining the relative proportions of each of the leaf foods in possum diets, for each individual, and using a repeated measures factorial ANOVA to determine significant differences in diet composition between the three diet treatments.

Hypotheses 2 and 3 were tested by quantifying the consumption rates of dry matter, digestible organic matter and available nitrogen per individual, for each diet, and comparing those daily means using factorial, repeated measures ANOVAs.

A target intake ratio for energy to protein was calculated for each diet separately, using the mean daily consumption rates of each nutrient.

Daily means were derived from five days of data collection for each of the three treatments. The number of individual possums was eight. The significance threshold used in this research was set at $p < 0.05$. All statistical analyses were performed in R 3.2.1.

5.4 Results

5.4.1 Variation in diet composition

M. australis was always eaten in the largest amounts relative to other foods (Figure 5.1). All species offered were consumed to some extent during each treatment, none were rejected outright (Figure 5.1). The entire mass of flowers offered to possums was always eaten, but only some of the pasture offered to possums was consumed, and observations were that the herbaceous species were picked out of the mixed sample and selectively consumed. Grass was consumed at a much lower frequency than it occurred in the sample.

There was a significant difference between the three diets in terms of the proportions of *F. excorticata* eaten ($F_{(2,14)}$ = 14.02, p = .001). Tukey post hoc testing revealed that when flowers were added to the diet, possums ate a significantly less *F. excorticata* than when consuming only the basal leaf diet. Adding pasture did not have a significant effect on the amount of *F. excorticata* eaten (Figure 5.1).

There was a significant difference between the three diets in terms of the proportions of *A. serrata* eaten ($F_{(2,14)}$ = 18.35, p = .001). Tukey post hoc testing revealed that when flowers were added to the diet, possums ate significantly less *A. serrata* than when consuming only the basal leaf diet. Adding pasture diet also significantly reduced *A. serrata* consumption. There was no difference in *A. serrata* consumption between the pasture and flower treatments (Figure 5.1).

There was a significant difference between the three diets in terms of the proportions of *M. australis* eaten ($F_{(2,14)}$ = 7.12, p = .001). Tukey post hoc testing revealed that when flowers were added to the diet the consumption of *M. australis* remained constant. However, when pasture was added to the diet, possums significantly reduced their consumption of *M. australis (*Figure 5.1).

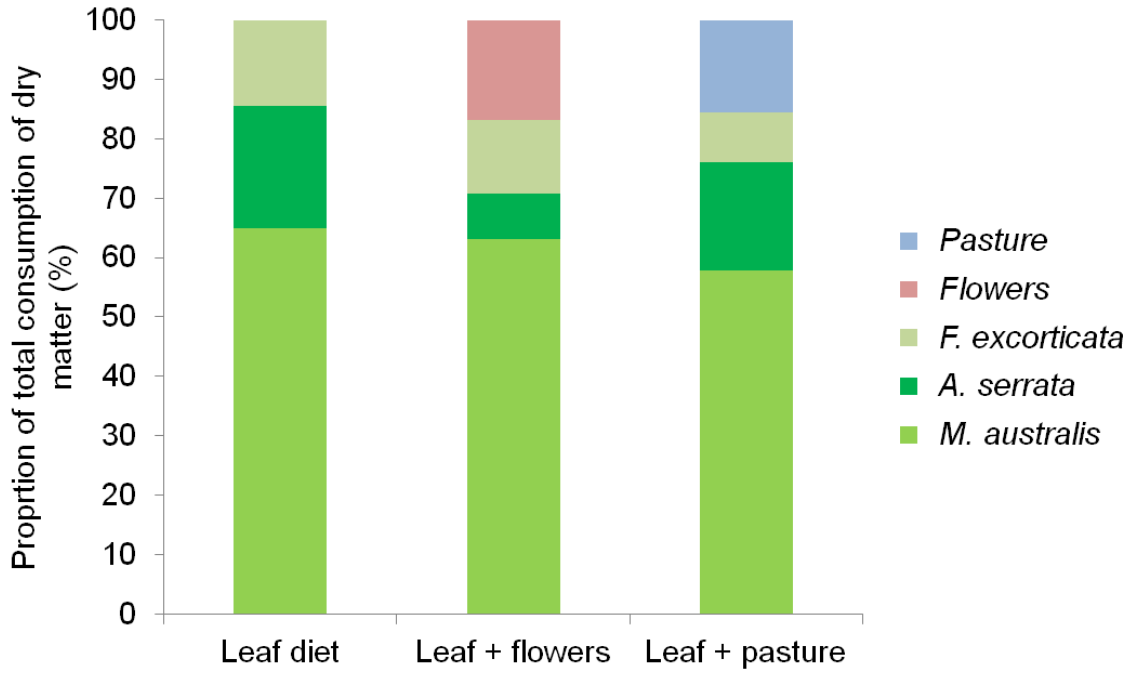

Figure 5.1: The proportional contribution of each food type offered to the total consumption (% of dry matter consumed) varied between the three diets (leaf only, leaf + flowers and leaf + pasture). Eight possums were observed and means were calculated from 5 days per treatment.

5.4.2 *Variation in the nutrient characteristics of foods offered*

There was a significant difference between the five foods in terms of both the available nitrogen content (% of dry matter) ($F_{(4,56)}$ = 38.11, p = .001) and the digestible dry matter content ($F_{(4,56)}$ = 19.19, p = .001) (Figure 5.2).

Tukey post hoc testing revealed that *M. australis* foliage and pasture had the highest available nitrogen contents, followed by *A. serrata* foliage. *F. excorticata* foliage and flowers had significantly less available nitrogen than the other food items. Post hoc testing also revealed that *A. serrata* foliage had the highest digestible dry matter contents, and *M. australis* had significantly lower digestible contents than any of the other food items (Figure 5.2).

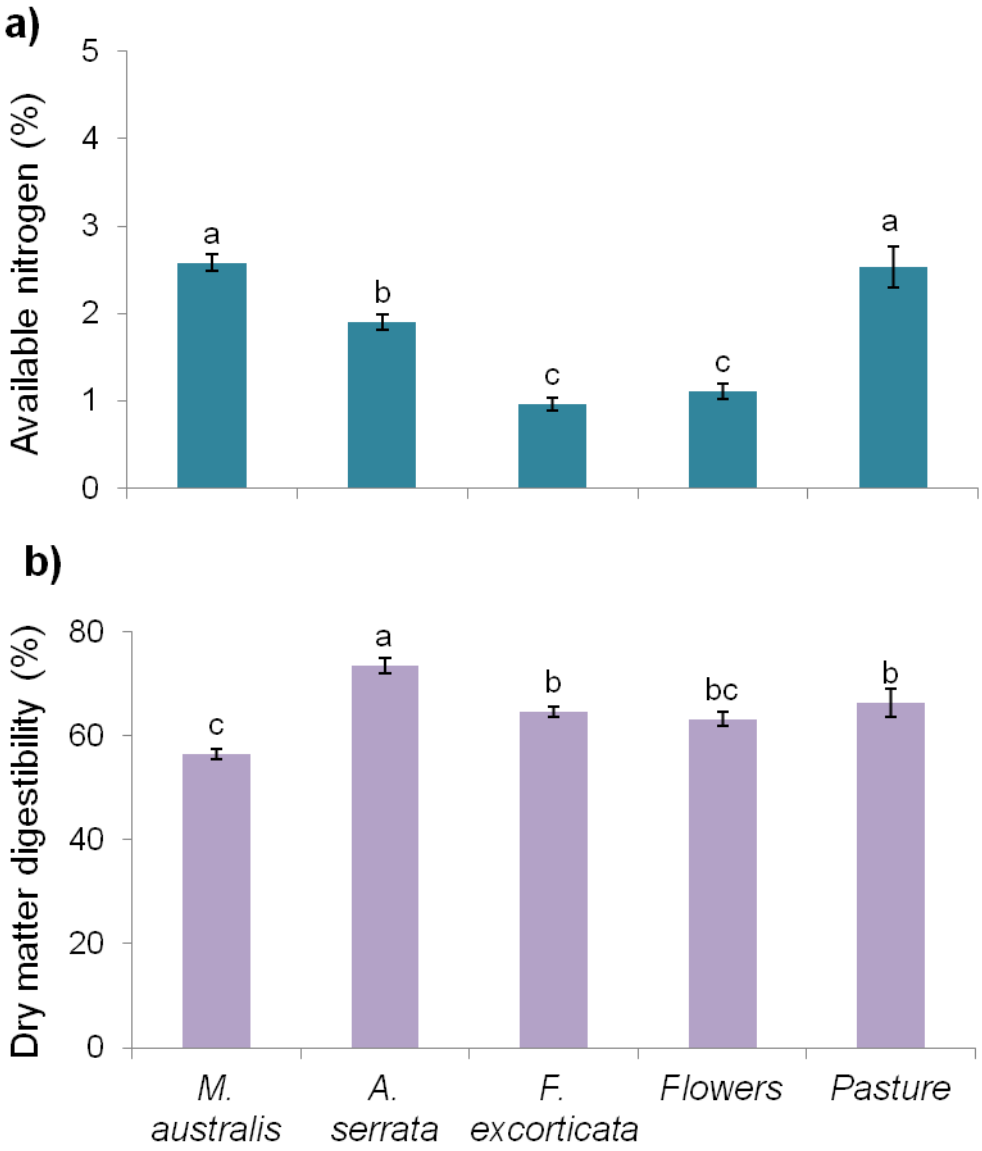

Figure 5.2: Nutritional characteristics of each of the five food items offered to possums during the feeding trial, which were *M. australis* foliage (n = 14), *A. serrata* foliage (n = 14), *F. excorticata* foliage (n = 14), flowers (n = 7) and pasture (n = 10). Foods are described according to a) available nitrogen and b) digestible dry matter contents (% of dry matter). Error bars show standard errors around the mean. Means that do not share the same letter are significantly different at $p < .05$.

5.4.3 Variation in dry mass consumption rate with diet

There was a significant difference between the three diets in terms of the consumption rate of dry matter (grams per day) ($F_{(2,14)}$ = 49.84, p = .001) (Figure 5.3a). Tukey post hoc testing revealed that when flowers were added to the diet, possums ate significantly less dry matter than when consuming only the basal leaf diet. Adding pasture did not have a significant effect on the amount of dry matter eaten compared to the basal leaf diet. When possums supplemented their diet with pasture they consumed significantly more dry matter than when they supplemented their diet with flowers.

5.4.4 Variation in energy consumption rate with diet

There was a significant difference between the three diets in terms of the consumption rate of digestible matter ($F_{(2,14)}$ = 43.04, p = .001) (Figure 5.3c). Tukey post hoc testing revealed that the consumption rate of energy didn't vary between leaf only and leaf + pasture, but was significantly less on leaf + flowers than on either of the other two diets.

5.4.5 *Variation in protein consumption rate with diet*

There was a significant difference between the three diets in terms of the consumption rate of available nitrogen ($F_{(2,14)}$ = 122.99, p = .001) (Figure 5.3b). Tukey post hoc testing revealed that the consumption rate of available nitrogen was highest on a leaf + pasture diet, followed by leaf only. Possums consumed significantly less available nitrogen when on a leaf + flowers diet than on either of the other two diets.

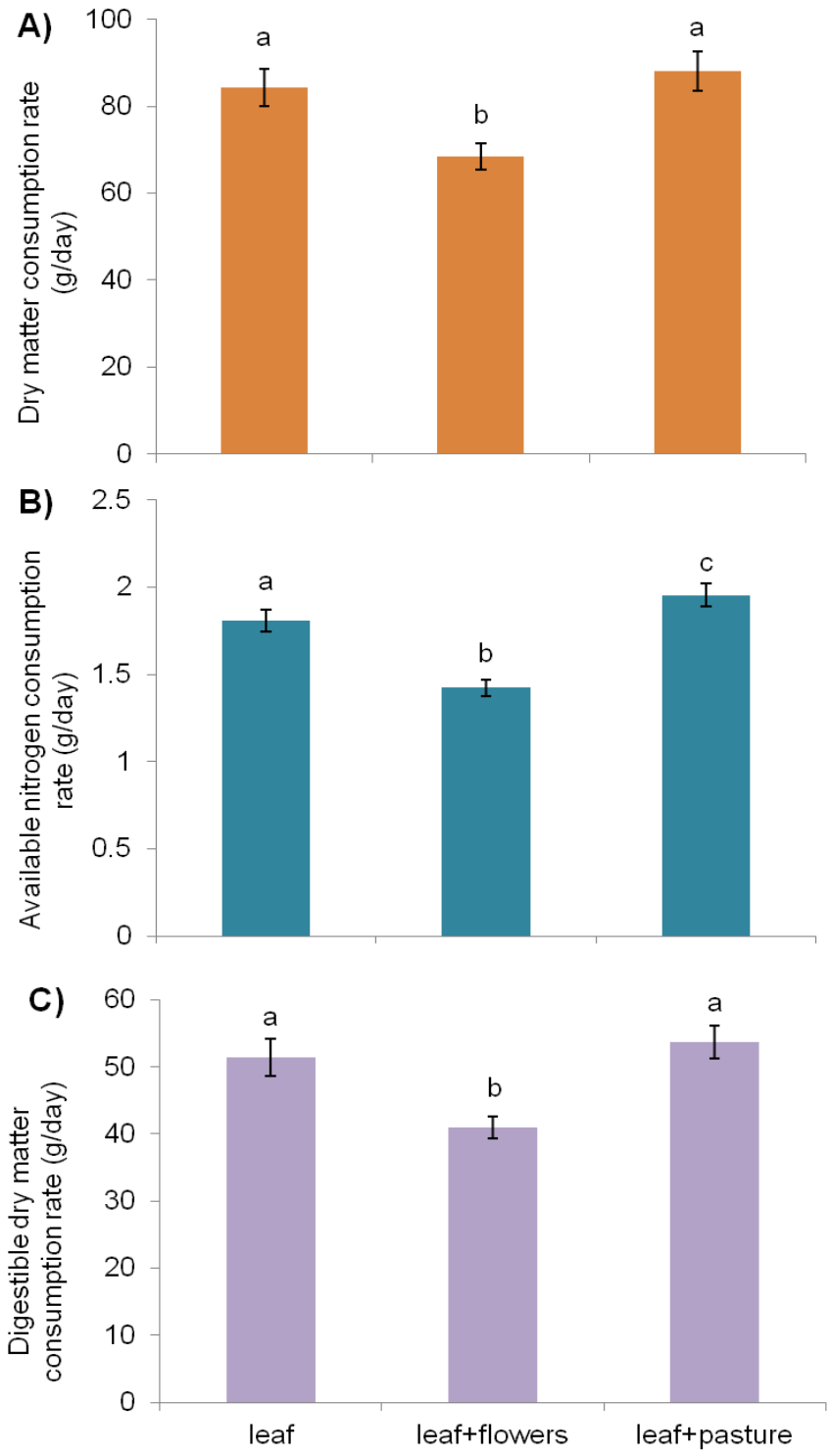

Figure 5.3: The differences in the consumption rates of A) dry matter, B) available nitrogen and C) digestible dry matter (g day $^{-1}$) when possums were fed the three different treatment diets (leaf only, leaf + flowers and leaf + pasture). Eight possums were observed and means were calculated from 5 days per treatment. Error bars show standard errors around the mean. Means that do not share the same letter are significantly different at $p < .05$.

5.4.6 Nutrient ratios

Each of the five food types fed to possums varied in term of the ratio of energy to available nitrogen it contained (Table 5.2).

The ratio of energy to available nitrogen intake rates is assumed to represent a nutritional target intake ratio for possums. Analysis of nutrient ratios during each of the three diet treatments showed that the target ratio of one to the other was the same on two of the diets (leaf and leaf + flowers) and very similar on the third (leaf + pasture) (Table 5.3).

Table 5.2: Ratio of mean digestible dry matter contents (g) to available nitrogen contents (mg) for each of the food types fed to possums during the feeding trial.

Food type	Ratio of digestible dry matter (g) **:** available nitrogen (mg)
Muehlenbeckia australis foliage	1 : 4.6
Aristotelia serrata foliage	1 : 2.6
Fuchsia excorticata foliage	1 : 1.5
Rosa spp. flowers	1 : 1.8
Pasture spp. foliage	1 : 3.8

Table 5.3: Mean daily consumption rates per treatment diet of digestible dry matter (g/day) and available nitrogen (g/day) and the resulting ratio of the consumption of one nutrient to the other (g dry matter : mg available nitrogen), taken to be the target intake nutrient ratio.

Diet treatment	Digestible dry matter (g/day)	Available nitrogen (g/day)	Ratio of digestible dry matter (g):available nitrogen (mg)
Leaf	51.35	1.81	1 : 3.5
Leaf + flowers	41.04	1.42	1 : 3.5
Leaf + pasture	53.69	1.96	1 : 3.7

5.5 Discussion

5.5.1 Variation in consumption

Possums altered their patterns of consumption when a basal leaf diet was supplemented with nutritionally different foods (i.e. pasture and flowers). Hypotheses 1 was supported: possums consumed foods in different proportions across three different treatment diets. Hypothesis 2 was supported because possums also consumed significantly different amounts of dry matter when feeding on each diet. Foods were found to have significantly different nutrient compositions and so the intake rates of both digestible dry matter (as a proxy for energy) and available nitrogen (as a proxy for protein) both varied significantly with diet, which supports hypothesis 3. A target nutrient intake was defined from the intake rates, as a ratio of the intake rate of protein to energy. This allows the discussion of the hypothesis testing in relation to the geometric framework.

5.5.2 Nutritional targets

When captive herbivores are fed mixed diets in captivity, it can be assumed that their food selection and subsequent nutrient intake rates are a result of unconstrained *ad libitum* dietary selection; these can then be defined as their intake target ratio (Simpson & Raubenheimer 2012; Felton et al. 2016). This study calculated nutritional target intake ratios in possums, and found that possums reached a consistent target across the three treatments (1:3.5, 1:3.4 and 1:3.7).

Other herbivorous mammals have been shown to have target intake rates when fed mixed diets in captivity. For example, moose (*Alces alces*) offered a free-choice, mixed diet did not consistently choose the foods containing the greatest amounts of energy or protein, but instead they combined foods in specific proportions to provide a particular ratio and amount of macronutrients: the target intake ratio expressed as energy (protein:energy) was 0.22 for both females (16:73) and males (20:93) (Felton et al. 2016). The authors of the study concluded that their findings lent further support to the geometric framework hypothesis (Felton et al. 2015). The results of this study further support that hypothesis because although intake rates varied, they reached a particular balance between macronutrients that was consistent across treatments. Furthermore, wild possums

do not consume the highest energy or highest protein foods that are available (Chapter 4). These results suggest that possums balance multiple nutrient intake rates and therefore the geometric framework is well-suited to use in further work to increase our understanding of their nutritional ecology. This study contributes to growing evidence that the geometric framework is the most robust theoretical model for assessing nutritional decisions herbivores (e.g. Felton et al. 2009; DeGabriel et al. 2014; Raubenheimer et al. 2014) and a range of other taxa, including humans (Simpson & Raubenheimer 2005; Köhler et al. 2012; Coogan et al. 2014).

5.5.3 Variation in intake rates

When flowers were used as dietary supplement possums were able to maintain the target ratio but consume less dry matter in total. In the wild this could be important because possums would have to consume less plant material for the same nutritional benefit. This could explain the strong selection for flowers when available. Flowers were not particularly high nitrogen foods, compared to *M. australis* for example; however, *U. europaeus* flowers are important dietary components during winter when deciduous leaves are unavailable.

Pasture consumption resulted in a slightly higher intake ratio of nitrogen to energy. But no overall change in amount of dry matter that was consumed to meet this target, relative to the leaf-only diet. The high levels of available nitrogen in foliage make pasture seem favourable, but other factors could influence these results. From personal observations during the feeding trial, possums did not consume the entire pasture sample offered to them. They selectively consumed the herbaceous species from the mixed sward sample, leaving the grass species behind. This selection suggests that something deters possums from feeding on grasses, the exact mechanism of which his beyond the scope of this study. One likely explanation is that grasses contain very different physical defences to those found in the leaves of woody plants: compared to ungulates, browsers are not as well adapted to the silica defences present in grasses. Therefore possums may be deterred from feeding on grasses. These results could help to explain the results of other chapters in which grass was not found in the stomach contents of possums at forest-pasture margins in Otago.

5.5.4 Relating laboratory findings to the field

Extrapolating results of laboratory studies to the field always presents difficulties because particular variables and conditions must be controlled during laboratory studies to enable more rigorous testing of experimental hypotheses. In this feeding experiment, only a small sub-set of the foods potentially available in the field were offered, mostly *ad libitum*. This is in contrast to the conditions experienced by wild herbivores, who must expend time and energy to search for food patches in a heterogeneous foraging environment. Despite the difficulties in extrapolating from laboratory experiments in artificial environments, such procedures do permit a more precise mechanistic approach to diet analysis that provides greater understanding of the underlying mechanisms driving diet selection by this species. These difficulties can be partly overcome if the results of feeding trials in the laboratory can be combined with analysis of stomachs from animals caught in their natural habitats, as in Chapter 3.

5.5.5 Foraging strategies

Laboratory and fieldwork results highlight the importance of a mixed diet and polyphagous feeding strategy in possums: *M. australis* foliage was always consumed in greater amounts than other foods. *M. australis* was higher in available nitrogen than all other foods except pasture, but had one of the lowest energy contents. If possums were to consume only *M. australis* they would not precisely reach the target ratio identified; they would have to over-consume nitrogen to reach their energy target. This would not be optimal because nitrogen cannot be stored, and there would be a cost of excretion. However, possums mixed their diets and consumed other, nutritionally different foliage during the same night: the foliage of *A. serrata* and *F. excorticata* had low concentrations of available nitrogen, but contained a relatively large amount of digestible organic matter (energy). Eating *A. serrata* or *F. excorticata* alone would have meant possums had to over-consume energy to reach their nitrogen target. Although energy can be stored, it would not always be optimal to do this in the wild. By consuming these foods as a mixed diet, possums were able to regulate the intake of multiple nutrients from a set of foods that if eaten in isolation would be nutritionally inadequate.

5.5.6 Foraging theory

Classical optimal foraging theory (Emlen 1966; MacArthur & Pianka 1966) predicts that animals will forage to maximise profitability in terms of energy per unit time. However, the results of these experiments do not support predictions from optimal foraging theory because neither energy nor nitrogen intake rates are maximised at the expense of other nutrient requirements, particularly nitrogen. This study shows that possums were able to mix their diets, switching between nutritionally different foods, whilst maintaining a target intake ratio of several nutrients. This helps to explain field results which found possums exhibiting a seasonally different polyphagous foraging strategy at forest-pasture margins (Chapter 3).

Utilising a mixed diet not only enables polyphagous herbivores to obtain a range of nutrients, but may also act to reduce the detoxification load that would result from consuming a lot of one type of plant secondary metabolites (Wiggins et al. 2006; Torregrossa & Dearing 2009). The effect of detoxification of plant secondary metabolites on possum diet width was investigated by Dearing & Cork (1999). Possums maintained a higher intake of food when allowed to select from two diets containing different profiles of secondary compounds (phenolics and terpenes) than when given access to the diets individually. Furthermore, possums in captivity have also been shown to reduce their overall consumption of dry matter and alter their feeding patterns in order to reduce the negative influence of plant secondary metabolites on intake (Wiggins et al. 2003). This demonstrates that a polyphagous foraging strategy in possums is likely to result from not only their multiple nutrient needs but also from the effect of anti-feedant compounds in the plant tissues they consume.

Apart from controlling for the effect of tannins in the available nitrogen assay (DeGabriel et al. 2008) the role of plant secondary metabolites was beyond the scope of this study. Furthermore, although nitrogen and energy are both considered to be important in determining foraging behaviour, the role of other macro- (e.g. phosphorus and potassium) and micro-nutrients may also be important, and certainly warrant consideration in future work. By unifying several nutritional measures using geometric models, the Geometric Framework is the most suitable theoretical framework with which to explain the multiple dimensions of herbivore foraging behaviour (DeGabriel et al. 2014). The ‘nutrient space’

under consideration can be extended by increasing the number of dimensions used to describe it. This is done by increasing the number of axis beyond the two nutrients I considered, to include for example, plant secondary metabolites and other nutrients likely to correlate with fitness (e.g. Behmer et al. 2002).

Geometric Framework theory would predict that possums will consume a mixture of foods in differing proportions when offered a mixed diet, in a manner that ensures that they meet their intake target for both nitrogen and energy simultaneously. For that reason it can be considered to be a suitable theoretical framework within which to consider our results. In a recent review, DeGabriel et al. (2014) states that the Geometric Framework not only represents a key milestone in our ability to link nutritional ecology laboratory studies with field studies, but is also the best available tool for identifying the limiting nutrients in an animal's diet. The Geometric Framework was helpful for interpreting the results of this study and also provides a useful framework for further investigation of the effects of a wider suite of plant chemistry measures on possum foraging behavior in New Zealand.

Chapter 6: Foraging pathway characteristics and habitat selection of the brushtail possum at forest-pasture margins in New Zealand.

6.1 Abstract

Improvements in the technology used to remotely obtain spatiotemporal location data mean that it is now possible to monitor many previously cryptic movements, often over relatively long periods of time, long distances or at a high rate of fix frequency. Because the scale and resolution of observations now match the resolutions of the behaviours in question, it is possible to address questions about nightly foraging pathways and habitat selection in foraging arboreal herbivores using animal-borne GPS devices.

In this chapter, GPS collars were fitted to seven individual possums at native forest-pasture margins in New Zealand. Sequential location data were collected every 15 minutes for several nights in two different seasons. In total, 93 foraging pathways were analysed. Multi-Scale Straightness Index (MSSI) values were calculated at each recorded location and points were divided into two behavioural states; area-restricted searching (MSSI < 0.5) and travelling between patches (MSSI >=0.5).

For each pathway, the total time and total length were measured and the speed of travel and time spent searching within patches was calculated. There was significant variation in pathway characteristics between seasons but not between male and female possums in any season. The mean total pathway length was significantly greater in winter than it was in autumn. Possums travelled faster in winter than they did in autumn. It is proposed that changes in the availability and spatial distribution of key resources may explain some of the seasonal variation between foraging behaviour in possums.

Possum locations were used to create home range estimates (defined as the 95% Utilisation Distribution) for each animal in each season. The availability of different

habitat types within the home range was quantified and compared with the frequency of use of those habitats, for searching and travelling behaviours separately. Possums displayed significant habitat selection for both behaviours but the strength of selection was greater when possums were searching within patches as opposed to travelling between them. Possums preferred native bush/forest and mixed shrub/scrubland for both travelling and searching. Pasture often comprised a large proportion of possum home ranges, but possums were not located in pasture as frequently as would be predicted from the availability alone. It is proposed that possums prefer native shrub/scrubland and secondary growth broadleaf forested areas because seral vegetation is frequent in these areas.

6.2 Introduction

6.2.1 Study background

In Chapter three, possum foraging strategies were considered on the basis of diet analyses, with a strong emphasis on seasonal dynamics of diet. These results were supplemented in Chapter five by controlled laboratory experiments to determine food preferences and ingestion rates. However, for a more complete understanding of foraging strategies it is also necessary to observe patterns of foraging behaviour at the landscape level, as perceived by the organism itself.

A home range can be defined as 'the area traversed by the individual in its normal activities of food gathering, mating and caring for young' (Burt 1943). Home ranges are products of the interaction between the changing availability and distribution of key resources in the landscape and an animal's understanding of its environment i.e. its cognitive map (Powell 2000; Borger et al. 2008).Possums live within home ranges that are comprised of a mosaic of different habitats which vary both spatially and seasonally in their favourability as foraging environments. In this chapter I build upon the understanding of possum foraging ecology and resource use obtained in previous chapters, by measuring and characterising the movement trajectories of foraging possums. This information is used to estimate possum home ranges and assess patterns of habitat selection from which I draw conclusions about possum resource use in relation to the nutritional favourability of habitat types at forest-pasture margins.

6.2.2 Large herbivore foraging behaviour

Foraging behaviour by large mammalian herbivores is expressed over a hierarchy of spatial and temporal scales from bites and steps within a patch, to movements that connect food patches and a range of activity modes that generate daily, seasonal and annual home ranges (Owen-Smith et al. 2010). Application of foraging theory to mammalian herbivores has until recently focussed primarily on fine-scale foraging behaviour e.g. decisions concerning diet and food selection. However, questions about foraging can be extended to movement behaviour expressed by foragers over larger spatial and temporal scales (Owen-Smith et al.

2010). Nevertheless, this requires that the temporal and spatial resolution of the observations match the temporal and spatial scale of the behaviour of interest.

Recent advances in GPS technology have undoubtedly increased our capacity to measure a range of previously cryptic animal movement behaviour (Hebblewhite and Haydon 2010; Urbano et al. 2010). Use of GPS technology allows recording of sequential movements over larger spatial and temporal scales than is possible using direct observations. Data can be collected at a high fix frequency, over long periods of time, with a degree of accuracy often < 30 metres (Tomkiewicz et al. 2010). GPS tracking devices have also become more inexpensive and therefore more widely available (Ropert-Coudert & Wilson 2005; Hooker et al. 2007). Thus, use of GPS technology has great potential to increase our understanding of foraging behaviour in large mammalian herbivores.

6.2.3 Measuring movement pathways

These recent technological advances mean that properties of movement trajectories obtained through use of GPS can now be used to classify and analyse animal behaviour (Shamoun-Baranes et al. 2012; Soleymani et al. 2014). Increases in data resolution mean that movement behaviour can be analysed at the level of the 'step' i.e. the straight-line movement between successive locations obtained on an animal, often only minutes apart (Turchin 1998; Morales et al. 2004; Nathan et al. 2008). Individual steps can be characterised based on speed, tortuosity and displacement to measure aspects of foraging behaviour (Patterson et al. 2008; Edelhoff 2016).

Foraging activity is commonly identified by 'area-restricted searching', defined as a sequence of short steps with frequent large turning angles between them (Benhamou 1992; Owen Smith et al. 2010). When foraging animals travel between patches they do so at higher speeds and in a more linear fashion than for movements within a patch (Owen Smith et al. 2010). Studying properties of individual steps along a trajectory means data are obtained at intervals small enough so that a single foraging bout within a patch, or a movement between patches, can be observed with several locations. Critically, this information can be used to differentiate behaviour within patches from travel to patches. Such data

can be used to answer questions about foraging behaviour and activity states along the movement pathway (e.g. Thiebault & Tremblay 2013; Alvard et al. 2015).

Postlethwaite et al. (2013) describe a new measure for analysing animal movement data, called the Multi-Scale Straightness Index (MSSI). The MSSI describes the ratio of the straight-line distance between the start and end of a movement to the total distance travelled (the straightness index) calculated repeatedly for track segments at all temporal scales, across a range of temporal scales. This allows for comparison among the different segments of trajectories resulting in the detection of changes in behaviour along a movement trajectory, over time and at different temporal resolutions. Nightly foraging pathways can be divided into two behavioural modes based on the MSSI value calculated at each recorded location: area-restricted searching (activity within a patch) and travelling between patches.

6.2.4 Habitat selection and foraging behaviour

The availability of spatial data has also created opportunities to advance methods that link behavioural modes to resource selection (Forester et al. 2009; Dzialak et al. 2015). Habitat selection studies assume that animals are more likely to be found in the most profitable or favourable areas of their home range and these areas must represent the optimal selection of resource units from those available. Most habitat selection studies measure resource use according to presence, regardless of the behavioural state of the animal at the time. However, polyphagous browsers forage in multiple patches and their home ranges are generated by both movements within foraging patches and movements between these areas. As a result, patterns of resource selection are likely to vary if locations associated with travelling and those associated with area-restricted movements are considered separately. Studies that use remotely sensed data to define behaviourally explicit resource selection are rare. When this has been considered, important differences have been identified which have had significant implications for species management (e.g. Zeller et al. 2014; Dzialak et al. 2015).

6.2.5 Specific 'gaps in the science'

GPS-derived location data are particularly useful for studying nocturnal, arboreal foragers such as the brushtail possum, on which collecting direct observational data at a high temporal resolution is practically impossible. Possums typically have 1-3 ha home ranges, utilise multiple den sites and may move several hundred meters in the course of a night's foraging, feeding polyphagously in the canopy and to a lesser extent on the ground (Cowan & Clout 2000). The difficulty of directly observing possums in dense canopy at night has resulted in a large gap in our knowledge of the activity patterns, search pathways and food patch use (i.e. foraging behaviour) of possums, and many other equivalent species, despite a strong practical need for this information (Cowan & Clout 2000).

Significant seasonal variation in the diet composition and foraging strategies of possums has been found in this habitat type (Chapter 3), but no difference between males and females was found. Previous studies have found that in some habitat types male and female possum diets do differ and studies on possum home ranges have shown intersexual differences. Despite this there has been no investigation of the influence of sex or season on the foraging pathway characteristics of possums.

The majority of previous studies of possum spatial ecology have been limited to estimating home range sizes (Jolly 1976; Green 1984; Ward 1984; Brockie et al. 1987; Paterson et al. 1995). Home range sizes have been quantified in different habitat types and at different possum densities in both Australia and New Zealand and have been useful in explaining and predicting the impacts of possums on native biodiversity (e.g. Innes & Barker 1999) and understanding their role in Tb transmission (e.g. Coleman & Caley 2000; Pech et al. 2010). However, direct comparisons between these studies are impossible due to wide discrepancies in the methods used to collect (e.g. live-trapping, spotlighting, radio tracking and GPS tracking) and analyse the data (e.g. different home range estimators such as Minimum Convex Polygons, Local Convex Hull, Utilisation Distribution, Brownian Bridges etc.). More recently, researchers have studied resource selection by brushtail possums within their home range (Whyte et al. 2013; Adams et al. 2014). However, the temporal resolution has not been fine enough to identify foraging patches or investigate foraging behaviour, despite a clear need for such

information. Furthermore, with one exception, previous possum studies using telemetry have all considered locations as independent of one another i.e. have not considered locations as a series of temporally autocorrelated points along a movement trajectory. This is despite the fact that they have in fact been collected sequentially. Lack of appropriate data has lead to a large gap in our understanding of possum foraging behaviour.

The approaches used to date that define a possum's space use presume that only one movement state exists, but in a foraging polyphagous folivore this cannot be the case. I hypothesise that in their search for and acquisition of resources possums are likely to display multiple behavioural modes (i.e. searching and travelling) and I need to understand how these activities are partitioned spatially or temporally. Characterising how possums move and forage according to season and sex, and identifying the key foraging habitats is vital to understanding the nutritional ecology of this species in its introduced range. Currently this kind of information is lacking, despite its potential use in informing pest management strategies and improving the efficiency of possum control operations. Such data are also needed to parameterise models to predict the impacts of possums at different densities and under a range of management conditions (Duncan et al. 2011; Glen et al. 2012).

6.2.6 Aims, objectives and hypotheses

In this chapter I describe landscape-scale foraging behaviour of brushtail possums at secondary forest-pasture margins using analysis of GPS location data. Movement pathways are segmented to distinguish between two behavioural states, i.e. 'area restricted searching' within patches and 'travelling' between patches, using the value of the Multi-Scale Straightness Index (MSSI).

Pathway characteristics are investigated in relation to variation between seasons and between sexes.

Pathway characteristics are defined as;

i. Total length of foraging pathway (metres)
ii. Total time spent active (minutes)
iii. Proportion of time spent in area restricted searching behaviour (%)

iv. Speed when travelling (metres/minute).

Recorded locations along foraging pathways are also used to define a theoretical home range (defined as the 95% Utilisation Distribution) for each individual possum. Habitat use is compared with habitat availability to test for and describe habitat selection by individual possums. Habitat selection is described separately for travelling and area-restricted searching behaviour.

The following hypotheses are tested:

H1: Pathway characteristics will vary between male and female possums.

H2: Pathway characteristics will vary between autumn and winter.

H3: Possums will display habitat selection when searching.

H4: Possums will not display habitat selection when travelling.

6.3 Methods

6.3.1 Trapping Possums and Fitting Collars

Possums were fitted with GPS transmitters during the winter of 2012 and the autumn of 2013 at Pigeon Flat; a remnant of broadleaf podocarp forest surrounded by a matrix of bush, shrub, scrub, and grassy/pasture habitats near Dunedin, New Zealand (Figure 2.3). Further details of the Pigeon Flat study site are given in Chapter 2. Work was carried out under The University of Otago Animal Ethics permit number AEC 65/12.

Cage traps were baited with apple; flour and cinnamon were used as lures. Once caught, possums were weighed in sacks then sedated for further handling. In order that the collars weighed no more than 5 % of the animal's body weight, possums less than 2.5 kg were not collared.

Possums were anesthetised with an intramuscular injection of Ketamine (100 mg ml^{-1}) and Medetomidine hydrochloride (1 mg ml^{-1}) and were individually marked with ear tags for identification in all subsequent trapping and re-trapping efforts (Web Ear Tags #3, Department of Conservation, New Zealand). Each possum was fitted with a 120g Wildlife GPS datalogger collar (SirTrack Electronics, Havelock North, New Zealand, http://www.sirtrack.com) which was equipped with a 12-channel GPS receiver Trimble iQ.

Collars were programmed to acquire and record the spatial location (longitude and latitude), date, time, quality of the location fix (horizontal dilution of precision), and the number of satellites from which the fix was acquired, at 15 minute intervals. Recording ceased when collars could no longer record a signal, i.e. when possums had returned to their dens. Recording began again the following night when possums came out from their dens. This enabled data to be collected over 7 to 11 consecutive days per individual (Table 6.1).

An intramuscular injection of Atipamezone hydrochloride (5 mg ml^{-1}) was used to reverse the effects of sedation and possums were released at the site of capture. Any GPS fixes obtained during the daytime hours on the day of their re-release were removed prior to analysis.

Four of the original five possums, were re-caught during the second season, and a further 2 were also collared in the second season. In total 7 unique individuals were tracked, 4 of these were collared twice, in different seasons; winter 2012 (2 male and 3 female), and autumn 2013 (2 male and 4 female) (Table 6.1).

6.3.2 Foraging pathway analysis

Sequential location data were collected every 15 minutes for several nights for each individual. In total, 93 foraging pathways were analysed. Pathways were characterised by their total length in space and time. The first GPS location of a possum nightly pathway was recorded when the GPS could receive and log a signal, i.e. when possums emerged from their dens. GPS collars stopped logging locations when the signal was interrupted i.e. possums had returned to their dens at the end of the night. The straight line distance between locations was measured, and summed to reveal the total length of the foraging pathway. The time between the first and last location in any one night was taken to be the total time spent active.

Each 'step' (consecutive location) along a foraging pathway was characterised using the Multi-Scale Straightness Index (MSSI) value, which was calculated at each recorded location (Postlethwaite et al. 2013). This resulted in points being divided into 2 behavioural states, based on the MSSI value; within patch activity (MSSI < 0.5) and between patch travelling (MSSI >=0.5). The proportion of the time spent within patches and the speed at which possums travelled between patches was then calculated. The proportion of time searching was the sum of time in which the value of the MSSI value was < 0.5 divided by the total time spent active. The speed of travel was calculated as the mean distance travelled divided by the time elapsed when MSSI values were >= 0.5.

Factorial ANOVAs were used to investigate the effects of season, sex and the interaction between season and sex on foraging pathway characteristics. The significance threshold used in this research was set at $p< 0.05$. All statistical, habitat selection and pathway analyses were performed in R 3.2.1 for Windows.

Unlike visual observations of activity budgets, GPS telemetry does not permit separating relatively stationary behaviours such as resting, sleeping, grooming, from other relatively sedentary feeding behaviour i.e. searching within patches. Hence I use the term 'within patch activity' to cover all these behaviours but in the belief that their proportions of activity in patches do not vary between patch so that within patch activity will correlate very closely with time spent feeding in a patch.

6.3.3 Home range and habitat selection analyses

Maps of possum locations were overlaid on a 2m x 2m raster map of habitat cover type (Fig 6.1) to evaluate habitat use by individual possums. Use was further subdivided, as before, into points at which possums were travelling and points at which possums were carrying out within-patch activities, based on the value of the MSSI. Thus habitat use is calculated for each behavioural mode separately.

Possum locations were used to create a home range estimate (defined as the 95% Utilisation Distribution) for each animal. The utilisation distribution was estimated using a biased random bridge approach (Benhamou & Cornelis 2010; Benhamou 2011), appropriate for animals with serial autocorrelation of the locations (using the R package 'adehabitatHR').

Home range contours were overlaid on the habitat map to quantify the available habitat for each possum separately (Fig 6.1). Habitat selection was assessed for each behavioural mode using Chi-square tests and Manly's Selection Ratios (Design III) both for each animal and the group overall, using the R package 'WidesIII'.

Manly's Design III selection ratios are applicable when both use and availability are measured and known for each individual (Manly et al. 1993). In this study, habitat use is defined as the proportion of points that occur in each habitat type and availability refers to the proportion of each habitat type within the home range. Where a habitat type was not used despite being available, it was assigned a value of 0.01% during analyses. For each animal, a resource selection index was calculated for each habitat: where values were greater than 1, the habitat was selected for; where values were less than one, habitats were selected against

based on the assumption that density was correlated with preference (Manly et al. 1993).

6.3.4 Description of habitat cover classes

The study site was centred on a fenced remnant of native broadleaf-podocarp forest surrounded by a matrix of forest, scrub and grassland habitats typical of farmland in lowland Otago (Figure 2.3). Possum home ranges within the study site were comprised of a mixture of 13 different habitat cover classes, which were:

Tree and forest habitats

1. **Broadleaf-podocarp forest:**

In New Zealand, broadleaf-podocarp forest is a complex, species-rich forest with a dense layered structure of emergent conifers (podocarps), a lower canopy of broadleaf trees, a range of vines and epiphytes and a dense ground cover of native ferns. At the study site the canopy was dominated by *Fuchsia excorticata* (fuchsia), *Melicytus ramiflorus* (mähoe) and *Griselinia littoralis* (broadleaf), with emergent *Prumnopitys ferruginea* (miro), *Dacrydium cupressinum* (rimu), *P. taxifolia* (mataï), and *Podocarpus hallii* (Hall's tötara). There is a dense understory that included *Pseudopanax crassifolius* (lancewood), *Raukaua simplex* (haumaköroa) and *Schefflera digitata* (patë).

2. **Bush forest:**

This habitat type is secondary-growth, low-canopy forest. Fast growing 'seral' species dominate. In particular; *Kunzea ericoides* (kanuka)*, F. excorticata, Aristotelia serrata* (wineberry) *Carpodetus serratus* (putaputaweta), *S. digitata*, *M. ramiflorus*, *Pennantia corymbosa* (kaikomako) and *Myrsine australis* (mapou). The understory is much less complex than in the broadleaf-podocarp forest, but *Coprosma* spp. are common as are the vines *Rubus cissoides* (bush lawyer) and *Muehlenbeckia australis* (muehlenbeckia)

3. **Kanuka/Manuka forest:**

Recently regenerating native scrub comprised of *K. ericoides* (kanuka) and *Leptospermum scoparium* (manuka).

4. **Pine plantation forest:**

Introduced *Pinus* spp. plantation forests.

5. **Shelterbelts:**

Introduced *Macrocarpa* spp. trees used as shelter belts.

6. **Tree group:**

This habitat was isolated native hardwoods that often formed minor shelter belts or ran parallel to farm tracks. As stock had access to these areas they were generally comprised of unpalatable species such as *Pittosporum eugenioides* (lemonwood) and *Pseudowintera colorata* (horopito) but also *K. ericoides*.

Shrub / scrubland habitats

7. **Mixed scrub/shrubland:**

A mixture of broadleaf shrubs/scrub species that tend to develop on moderately fertile and fertile soils. At our study site this habitat was comprised primarily of *F. excorticata, M. ramiflorus*, *Pseudopanax arboreus* (five-finger), *Brachyglottis repanda* (rangiora) and *Hebe salicifolia* (koromiko). Native broadleaf shrub/scrub species were mixed with introduced scrub species, particularly *Ulex europaeus* (gorse). *M. australis* was particularly common in recently disturbed areas and on the margins.

8. **Gorse / Broom scrub:**

Scrubland comprised of introduced *U. europaeus* and *Cytisus scoparius* (broom).

Grassland habitats

9. **Pasture:**

High-producing, improved pasture containing the grasses *Agrostis capillaris* (browntop), *Anthoxanthum odoratum* (sweet vernal), *Lolium perenne* (ryegrass), *Dactylis glomerata* (cocksfoot) and *Holcus lanatus* (Yorkshire fog), as well as clovers (*Trifolium* spp.), daisy flatweeds (e.g. dandelion, catsear, hawkbit and daisies), and other herbs.

10. **Rough grass:**

Patches of unimproved, low producing grassland.

Other habitats

11. **Bare ground**
12. **Built-up** (roads and houses)
13. **Standing water** (stock ponds).

6.4 Results

6.4.1 Home range size

Analysis of GPS data collected from possums showed a large variability in the home range size (Table 6.1). The overall mean home range size for all possums was 4.24 ha (SE ± 0.94 ha). The range for males and females was similar: from 0.97 ha to 9.41 ha (mean = 5.33 ha; median = 5.48 ha; SE ± 2.00 ha) for males, to 1.10 ha to 8.40 ha (mean = 3.62 ha; median = 2.71 ha; SE ± 0.99 ha) for females. Home range size differed between seasons: the median in winter (season 1) was 1.82 compared to 4.86 in autumn (season 2) and the mean increased from 3.04 ha (SE ± 1.60 ha) in winter to 5.24 ha (SE ± 1.03) in autumn.

Table 6.1: Details of possums collared at forest-pasture margins in Otago, New Zealand (sex, mass and season), their home range sizes, and the dates and number of nights of observation the results are based upon.

Individual	Sex	Season	Mass (kg)	Home range size (ha)	Number of nights of observation	Dates of observations
A	M	1	3.4	9.41	7	30/04/12 - 06/05/12
		2	3.7	2.99	8	02/03/13 – 09/03/13
B	F	1	3.4	1.90	11	29/04/12 – 09/05/13
		2	3.5	5.79	11	27/02/13 – 09/03/13
C	F	1	2.5	1.82	10	02/05/12 – 11/05/12
		2	2.9	8.40	10	08/03/13 – 17/03/13
D	F	1	2.6	1.10	7	03/05/12 – 09/05/12
		2	3.1	3.59	7	10/04/13 – 16/04/13
E	M	1	3.9	0.97	10	29/04/12 – 08/05/12
F	F	2	3.2	2.71	8	01/03/13 – 08/03/13
G	M	2	2.9	7.96	11	29/03/13 – 08/04/13

6.4.2 Foraging pathway characteristics

Foraging pathway characteristics were analysed over 7-11 nights per individual (Table 6.1). Season had a significant effect on the total pathway length of brushtail possums (Table 6.3): Mean total pathway length was significantly greater in winter (686.2 m) than it was in autumn (494.6 m) (Figure 6.1).

Season also had a significant effect on the speed of travel (Figure 6.1, Table 6.3). Possums travelled faster in winter (2.4 m/s) than they did in autumn (1.8 m/s) (Table 6.2).

Some pathways characteristics were consistent between seasons; Total time active did not vary significantly with season (Table 6.3) and was 7.5 hours in autumn and 7.2 hours in winter (Table 6.2). Proportion of time searching also did not vary significantly between autumn and winter (Table 6.3), being 46.9 % and 54.2 % respectively (Table 6.2), although with a p-value of 0.066 this difference was close to being significant.

These seasonal effects were consistent between sexes (i.e. no interaction), and there was no significant effect of sex on any of the pathway characteristics (Table 6.3).

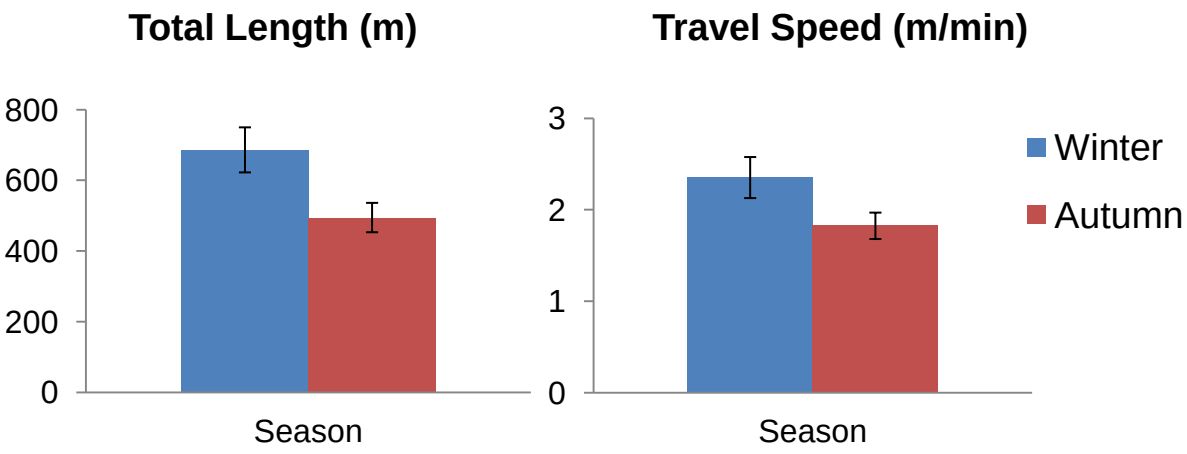

Figure 6.1: Significant differences in mean total length of foraging pathways (± SE) and the speed at which possums travelled (± SE), between autumn (red) and winter (blue), at forest-pasture margins in Otago, New Zealand.

Table 6.2: Summary of the pathway characteristics of brushtail possums at forest-pasture margins in Otago, New Zealand. Means and standard errors were calculated according to season (winter n = 5, autumn n= 6) and sex (male n = 5, female n= 7).

Pathway Characteristic	**Male**		**Female**		**Winter**		**Autumn**	
	mean	se	mean	se	mean	se	mean	se
Total length (m)	**621.3**	60.8	**553.6**	47.6	**686.2**	63.7	**494.6**	41.6
Total time (hours)	**7.5**	0.5	**7.2**	0.4	**7.2**	0.4	**7.5**	0.5
Proportion time searching (%)	**47.5**	3.0	**51.5**	2.6	**54.2**	3.3	**46.9**	2.3
Travel speed (m/s)	**1.9**	0.2	**2.2**	0.2	**2.4**	0.2	**1.8**	0.1

Table 6.3: The effect of season, sex, and season:sex on foraging pathway characteristics of brushtail possums at forest-pasture margins in Otago, New Zealand assessed by factorial ANOVA. * denotes a significant result.

Pathway Characteristic	**Season**			**Sex**			**Season:Sex**		
	F	df	p-value	F	df	p-value	F	df	p-value
Total length (m)	6.84	1, 88	0.011 *****	0.67	1, 88	0.416	1.44	1, 88	0.233
Total time (hours)	0.25	1, 88	0.618	0.14	1, 88	0.707	1.40	1, 88	0.240
Proportion time searching (%)	3.46	1, 88	0.066	1.11	1, 88	0.295	0.73	1, 88	0.396
Speed of travelling (m/s)	4.37	1, 88	0.039 *	1.50	1, 88	0.225	3.59	1, 88	0.062

6.4.3 *Habitat selection*

Most possums displayed significant habitat selection. The use of habitats for both travelling and searching by possums did not reflect the availability of the habitat within each individual's home range (Table 6.4). Selection for particular habitat types was greater when carrying out area restricted searches as opposed to when they were travelling between search patches (Table 6.4).

The overall patterns of habitat selection were mostly consistent between the two behavioural states. Overall habitat selection ratios showed that for both searching and travelling behaviours two key habitats were preferred; bush/forest, followed by mixed shrub/scrubland (Table 6.5). Within home ranges overall, selection for broadleaf forest was, on average) equal to its availability (Table 6.5).

Non-preferred habitat was also mostly consistent between the two behavioural states. Some habitats were theoretically available, but not used by possums. Other habitats were used for searching and/or travelling but to a lesser extent than would be predicted by their availability, so were selected against, or not preferred (Table 6.5). Possums selected against pasture, bare ground, exotic forest and tree groups during both searching and travelling behavioural modes (Table 6.5).

Table 6.4: Possums display significant habitat selection for searching and travelling behaviours at forest-pasture margins in Otago, New Zealand, evaluated using Chi-square test of overall habitat selection.

	X^2	df	p-value
Searching	279.7	63	.001
Travelling	218.6	63	.001

Table 6.5: Manly's Selection Ratios (with SE) demonstrate which habitat types are selected for and against when possums search and travel at forest-pasture margins in Otago, New Zealand.

Selection	Searching			Travelling		
	Habitat type	Selection Ratio	SE	Habitat type	Selection Ratio	SE
Preferred	Bush/Forest	2.00	0.74	Bush/Forest	2.21	0.42
	Mixed Scrub/Shrubland	1.69	0.25	Mixed Scrub/Shrubland	1.49	0.19
Equal use and availability	Broadleaved Indigenous Hardwoods	0.99	0.13	Broadleaved Indigenous Hardwoods	1.09	0.09
	Rough Grass	1.06	0.78			
Not preferred	Tree Group	0.78	0.27	Exotic forest	0.73	0.20
	Pasture	0.50	0.08	Gorse/Broom	0.65	0.34
	Exotic forest	0.38	0.14	Tree Group	0.49	0.25
	Bare Ground	0.29	0.15	Rough Grass	0.46	0.34
	Built-up	0.11	0.13	Pasture	0.44	0.07
				Bare Ground	0.33	0.19
Not used (close to zero ratio)	Kanuka/Manuka	0.01		Kanuka/Manuka	0.01	
	Pine plantation	0.01		Pine plantation	0.01	
	Gorse/Broom	0.01		Built-up	0.01	
	Standing water	0.03		Standing water	0.03	

Table 6.6: Most individual possums display significant habitat selection for searching and travelling behaviours at forest-pasture margins in Otago, New Zealand, evaluated using chi-square tests. * indicates a significant result.

Individual	Season	Searching			Travelling		
		X^2	df	p-value	X^2	df	p-value
A	1	11.21	5	.047 *	14.30	5	0.014 *
B	1	6.96	4	.138	8.96	4	0.069
C	1	13.17	3	.004 *	14.31	3	0.003 *
D	1	66.40	6	.001 *	27.85	6	0.001 *
E	1	32.40	8	.001 *	24.54	8	0.002 *
A	2	6.86	4	.143	3.71	4	0.447
B	2	18.33	6	.005 *	10.43	6	0.108
C	2	66.73	12	.001 *	64.88	12	0.001 *
D	2	35.03	7	.001 *	23.20	7	0.002 *
F	2	7.74	4	.101	10.19	4	0.037 *
G	2	14.82	4	.005 *	16.23	4	0.003 *

Table 6.7: Habitat composition of possum home ranges at forest-pasture margins in Otago, New Zealand. The corresponding searching behaviour selection index for each habitat type and for each individual is displayed in brackets. Green shading indicates positive selection. Light green shading indicates approximately equal selection. Habitats with a zero ratio were present in the home range but not used as search areas for possums. Grey shading indicates possums that did not display significant habitat selection while searching.

Individual	Season	Bare Ground	Native forest	Built-up	Bush/ Forest	Gorse/ Broom	Kanuka/ Manuka	Mixed scrub/ shrub	Exotic forest	Pasture	Pine plantation	Rough grass	Water	Tree group
A	**1**	0.08 (0.1)	60.94 (0.9)					16.33 (2.0)	2.33 (0.0)	19.20 (0.7)				1.12 (0.0)
B	**1**	0.02 (0.5)	64.20 (0.8)					20.60 (1.7)		11.89 (0.8)				3.29 (0.3)
C	**1**		65.31 (1.3)					17.93 (0.4)		13.29 (0.2)				3.46 (0.5)
D	**1**	2.67 (0.3)	46.05 (0.2)			6.27 (0.0)		14.62 (3.8)	0.23 (0.0)	28.15 (1.1)				2.02 (3.0)
E	**1**	2.88 (0.9)	8.34 (0.3)		0.96 (0.0)			23.53 (2.5)	3.63 (0.5)	57.97 (0.5)		0.08 (0.1)	0.11 (0.0)	2.51 (0.7)
A	**2**	0.80 (0.0)	62.42 (1.0)					28.85 (1.3)		7.82 (0.1)				0.11 (0.1)
B	**2**	1.63 (0.0)	46.81 (1.6)	1.01 (0.6)				21.03 (0.8)	3.11 (0.4)	24.23 (0.3)				2.18 (0.0)
C	**2**	2.41 (0.0)	0.33 (0.0)	5.02 (0.0)	12.04 (3.7)	3.35 (0.0)	2.59 (0.0)	18.34 (1.8)	3.47 (0.9)	42.10 (0.3)	4.92 (0.0)	2.27 (2.1)	0.54 (0.0)	2.59 (1.2)
D	**2**	0.90 (0.0)	4.94 (0.0)		27.48 (1.3)			21.53 (2.3)	10.91 (0.2)	29.46 (0.4)		2.23 (0.0)		2.55 (0.7)
F	**2**	3.04 (0.3)	50.66 (0.8)					27.36 (1.5)		18.83 (0.8)				0.12 (13.7)
G	**2**		72.15 (1.3)					8.10 (0.3)	1.01 (1.3)	16.06 (0.2)				2.68 (0.3)

Habitat selection is also demonstrated at the individual level (Table 6.6). Seven possums displayed significant habitat selection when both searching and travelling and two possums displayed significant selection when either searching or travelling. Only two possums did not display significant habitat selection within their home ranges during the study period (Table 6.6).

The patterns of habitat selection varied between individuals (Table 6.7). Native forest, bush/forest and scrub/shrub were the dominant available habitat types: they were present in all the possum home ranges. Results highlight the importance of scrub which was present in the home ranges of all individuals and selected for by the majority of individuals (Table 6.7). Bush forest only occurred in three home ranges but was positively selected by two of these possums. Results also show strong positive selection for native broadleaf-podocarp forest by several individuals. In this study broadleaf-podocarp forest commonly comprised approximately 50 - 75 % of the home ranges of possums (Table 6.7). Only one possum had a large proportion of native forest within the home range but selected against it.

Pasture was a dominant habitat type in the landscape, and was often a relatively large component of the estimated home ranges of possums. It comprised anywhere between 8 and 58% of home ranges, and was frequently used by possums, but often less than would be expected based solely on its availability (Table 6.7). This indicates a lack of strong preference for pasture habitat for foraging possums. However, there was variation between individuals in terms of their habitat selection. Lack of preference for pasture did not apply to individual D for example (Table 6.7). Furthermore, some possums selected for forest over scrub (e.g. individual C in season 1 and individual B in season 2) whilst others demonstrated preference for scrub over forest (e.g. individuals C and D in season 2 and also individuals A, D and E in season 1) (Table 6.7).

Bare ground (a pig field) was present in the majority of home ranges, as was exotic forest, but neither was a preferred habitat type for foraging possums (Table 6.7). It is not possible to comment on possum use of some habitats as they only made up a very small proportion of the habitat considered as available to possums (Table 6.7), therefore quantifying possum use of pine plantation forests, kanuka scrub and gorse/broom scrub is beyond the scope of this study.

6.5 Discussion

6.5.1 Home range size

The size of home ranges measured in this study was generally larger than those found in two other studies in similar habitat (pasture/scrub/remnant forest) (Table 6.8; Jolly 1976; Paterson et al. 1995). However, differences in methods used to collect locations (e.g. trapping, radio-tracking, GPS-tracking), and differences in the way the locations were computed into home rage estimates (e.g. Minimum Convex Polygons, Kernel Density Estimates or Utilisation Distributions), mean these kinds of comparisons may not be robust in all cases. For example, Jolly (1976) used a minimum area method from a pooled sample of locations collected using trapping, spotlighting and radio telemetry. In contrast, I employed a biased-random bridge approach, a method which takes into account not only the position of the relocations, but also the path travelled by the animal between successive relocations in calculating the utilisation distribution of the animal (i.e. a probability distribution of being located at any given location). This makes it hard to compare my results on home range size with many other studies.

Home range size varied between individuals and between individuals between seasons. Intrinsic factors (such as population density, age and reproductive status) and extrinsic factors (such as predator densities and food availability) have all been shown to influence possum home range size (Cowan & Clout 2000; Richardson et al. 2017). Variation between individuals is likely to be another intrinsic factor influencing home range size in mammalian herbivores (van Beest et al. 2011). As a result of the pest status of possums, much research in New Zealand has attempted to understand the variation in home range size; and in particular, to separate the effects of population density and habitat quality. In general, larger home ranges have been attributed to food resources being more widely dispersed in the environment, but in areas of low population density, encounters with other possums (i.e. for breeding) may be just as important as encounters with food items. Possums have been show to increase their home range size when population density is decreased through control (Efford et al. 2000). Furthermore, other studies have shown that reducing population density through control does not reduce the possum-to-possum contact-rates or breeding rates in the surviving population (Ramsey et al. 2002). Therefore this suggests that

home range size is likely to be influenced directly by population density, but also by food availability through its influence on population density (Cowan & Clout 2000).

Table 6.8: Home range sizes of possums in different habitat types in Australia and New Zealand generated using a range of methods (T = trapping; R = radio-tracking; G = GPS tracking; S = spotlighting). Based on tables produced in Cowan & Clout (2000); Rouco et al. (2013); Adams et al. (2014). Where one estimate is provided home ranges were not analysed separately for the sexes.

		Mean home range (ha)			
	Habitat	**Female**	**Male**	**Method**	**Reference**
New Zealand	Urban	2.4	4.9	G	Adams et al. 2014
	Modified forest, urban	2.7	1.5	T	Winter 1963
	Pasture/scrub/remnant forest	0.9	1.4	R	Paterson et al. 1995
	Pasture/scrub/willows	31.0	29.9	R	Brockie et al. 1987
	Pasture/scrub/remnant forest	0.9	3.1	T	Jolly 1976
	Mixed farmland/Beech forest	6.0		R	Ball et al. 2005
	Dryland	3.3	7.1	R	Glen et al. 2012
	Grassland/shrubland	36.2 – 54.1		T	Rouco et al. 2013
	Pine plantation	0.7	0.7	T	Warburton 1977
	Pine/Scrub/Forest areas	1.3	1.9	T	Triggs 1982
	Northofagus forest	3.1		G	Pech et al. 2010
	Podocarp/broadleaf forest	0.5		G	Blackie et al. 2011
	Podocarp-mixed broadleaf forest	0.5	0.8	T+S+R	Crawley 1973
	Podocarp-mixed broadleaf forest	2.6	3.9	R	Ward 1978
	Podocarp-mixed broadleaf forest	18.3	24.6	R	Green & Coleman 1986
Australia	Urban	1.9	10.9	T+R	Statham & Statham 1997
	Urban	1.02	1.2	R	Harper 2005
	Urban, wooded grassland	1.1	3.0	T	Dunnet 1956
	Eucalyptus woodland	0.9	1.1	T	Kerle 1984
	Open *Eucalyptus* forest	1.7	3.7	T	Winter 1976
	Eucalyptus forest	1.8	6.3	R	Allen 1982
	Eucalyptus forest	4.7	7.4	T	How 1981
	Eucalyptus rain forest	4.2	7.0	S	Hocking 1981

6.5.2 Effect of sex on foraging pathway characteristics

Differences in fundamental nutrient requirements and/or dietary preferences could result in different foraging tactics for males and females. Hence, it was predicted that males and females would have different foraging pathway characteristics, at least in some seasons. For example, if males had greater requirements for nutrients they could increase their intake rate by either increasing the proportion of time spent within patches (as opposed to inter-patch travelling), or increase their total time active. However, this study did not support a significant effect of sex any foraging pathway characteristics. Whyte et al. (2013) also did not detect inter-sexual differences in possums home range characteristics. However, other studies have found significant differences between male and female home range sizes, and associated range lengths (Jolly 1976; Crawley 1973; Blackie 2004; Adams et al. 2014). My results could be explained by the broad similarities in body size and weight between male and female possums (King 2005). Sex differences in herbivore dietary requirements and foraging behaviours have primarily been found in species that display significant sexual size dimorphism (e.g. bighorn sheep *Ovis canadensis* (Eruckstuhl 1998) and giraffe *Giraffa camelopardalis* (Ginnet & Demment 1997)).

Furthermore, results in Chapter 3 suggested that male and females did not differ in terms of diet selection or foraging strategy at forest-pasture margins. This could explain the similarities in foraging pathway characteristics found in this study, both in terms of distances and speeds travelled and the relative proportions of time spent either foraging or travelling. Conversely, the lack of intersexual variation in foraging pathway characteristics could simply be a result of the small sample size in this study, which means it cannot be considered to be an exhaustive analysis. It is also possible that significant intersexual variation in foraging pathway characteristics could occur in spring or summer; seasons that were outside the scope of this study.

6.5.3 Effect of season on foraging pathway characteristics

It can be predicted that possum foraging pathway characteristics would vary with season because the availability of foods within the landscape and the composition of possum diet are both significantly influenced by season (Chapter 3). The hypothesis that season would also influence foraging pathway characteristics was supported by the results: the total distance travelled by possums, and the speed at which they travelled between foraging areas, was less in autumn than it was in winter.

Larger home range areas (implying an increase in pathway length) have previously been associated with possums living in open habitats (e.g. Rouco et al. 2017). This is often been attributed to possums having to travel longer distances to find widely scattered resources, such as food, shelter or conspecifics (Sweetapple unpubl.). Movement is energetically costly therefore possums would be expected to minimise the distances they have to travel in order to meet their nutrient requirements. This could explain the significant seasonal variation in total distances travelled found in this study; i.e., if preferred food resources have a wider spatial distribution in winter and are more concentrated within the home range in autumn. It could be argued that when trees within a home range are fruiting in autumn there is an increase in availability of preferred foods within the home range, thus helping to explain the results I found. Possum diet is known to vary strongly with season, and this is likely due to changes in the favourability/quality and availability of different food items.

Possums consume twice as many different food items in autumn as they do in winter (Chapter 3). Most of this increase is due to fruit consumption from a wide range of tree species. In addition, the deciduous vine *Muehlenbeckia australis* is a highly preferred species in autumn, but is not eaten in winter because it is deciduous and therefore unavailable (Chapter 3). These two factors could help to explain some of the variation between these two seasons in terms of foraging pathway characteristics, because where the probability of encountering a preferred

item is higher, the distance travelled between foraging patches should be shorter, reducing the overall distance travelled.

Yockney (2015) also found variation in possum foraging behaviour with season. They used GPS location data and showed that foraging range lengths of possums in semi-arid montane areas of New Zealand were largest in winter and in spring and smallest in summer. The authors concluded that this was possibly due to a reduction in the availability and quality during winter which required animals to move over larger areas to meet their nutritional requirements. Studies on other herbivorous mammals came to similar conclusions, e.g. Elk *Cervus elaphus* (Anderson et al. 2005) and Mountain hares *Lepus timidus* (Kauhala et al. 2005).

The speed at which possums travelled between searching patches was greater in winter than it was in autumn. Speed choice is driven by a need for resources (e.g. food and conspecifics) but is constrained by intrinsic (e.g. energetic costs) and extrinsic factors (e.g. risk of injury or death) (Wilson et al. 2015). High speeds are energetically costly, so it could be argued that the nutritional benefits associated with faster speeds must outweigh those costs. Increased speeds also reduce an animal's ability to observe the environment (Wilson et al. 2015). Therefore, possums must move at a speed suited to detecting objects in the environment, in particular, for food resources because possums have no natural predators in New Zealand.

Spatial memory allows herbivores to revisit nutrient-rich sites and avoid low quality sites (Searle & Shipley 2008). In addition, visual clues can be used by browsing herbivores to predict and locate forage resources from a distance (Searle & Shipley 2008). Larger patches are more detectable than small or widely distributed food items (Laca & Ortega 1995). This could explain why possums (that are known to consume a high proportion of fruits in autumn) travelled slower compared to possums consuming mostly foliage in winter: because to detect fruits using visual clues requires slower speeds than those that are optimal for travelling between trees to consume foliage. The location of fruiting trees is likely to be more difficult for possums to predict based on their spatial memory as opposed to the location of trees generally. This would make visual clues even more important for fruit

consumption in autumn, which could further act to reduce the optimal speed of travel, compared to winter.

However, a mechanistic explanation of differences in speeds between autumn and winter may also contribute to the observed differences. Possums travel faster in winter in order to cover longer distances: possums were not observed to increase the total time they spent active, nor did they reduce the amount of time spent searching relative to travelling in order to cover the increased distances observed in this study.

6.5.4 Habitat selection at forest-pasture margins

It was hypothesised that possums should display strong habitat selection when foraging within patches, because some habitats will be more nutritionally favourable than others, depending on the availability and distribution of key food items. It was also hypothesised that possums would not display selection for any particular habitats when travelling. Findings of this study supported only the first hypothesis because the majority of possums displayed significant habitat selection both for travelling and searching behaviours, although selection for particular habitat types was stronger when searching.

The significant habitat selection when travelling could be explained by foraging patches being clumped in favourable habitats, as opposed to randomly through the home range, meaning that more of the travelling was within a habitat rather than between habitats. It may also be that some habitats were more favourable to travel through than others. Tree and shrub habitats were favoured for travelling compared with ground layer dominated habitats or dense scrub. The selection for tree and shrub habitats for travelling could be at least partially explained by the primarily arboreal habits of possums (King 2005). Possums may be more exposed to particular types of predators (either due to being more detectable or having nowhere to escape from predators) in open areas away from dense forest. Thus selection against open grassland habitats may be an artefact of possum's evolutionary history in Australia, where predator avoidance has been shown to play a key role in determining possum foraging behaviour and habitat use (Nersesian et al. 2011; Pickett et al. 2005).

Three possums did not display significant habitat selection, and there was also variation in which habitats were preferred by possums. In particular, some possums preferred scrub and selected against native forest whilst others selected for native forest and against scrub. These differences between individuals in terms of their movements and habitat selection patterns could be a result of a variety of biotic and abiotic factors that relate to static and dynamic environmental conditions and the state of the animal (Raynor et al. 2017). Future work could explore in more detail how possum movement strategies vary among individuals and years in response to both within- and between-season changes in environmental conditions.

In some previous studies in New Zealand possums have been shown to travel up to 1 km from inside forest to feed out on pasture (Green & Coleman 1986; Blackie 2004). However, the importance of pasture as a foraging habitat was not supported in many others studies (e.g. Jolly 1976; le Mar & McArthur 2005). Possums have previously been shown to select for shrub or forest over open or grassy areas (le Mar & McArthur 2015; Rouco et al. 2017) and habitat selection studies often describe pasture as not preferred (e.g. Jolly 1976). Despite this, possum home ranges in this study had a relative large amount of pasture within them and possums were frequently detected both travelling and searching in this habitat type, despite using it less than might be predicted from its availability alone. Pasture grass and herb mixes have high concentrations of nitrogen and readily soluble carbohydrate, and based on their nutritional composition alone should be a favourable food source, certainly comparable to the foliage of many of the broadleaf tree and shrub species known to be consumed by possums (Chapter 4). Furthermore, captive possums are shown to consume pasture species alongside tree foliage. This further reinforces the suggestion that there may be a nutritional basis for use of pastures by possums.

Possum searching within patches was focussed on three key habitat types; bush/forest, mixed shrub/scrubland, and broadleaved indigenous hardwood forest. That possums selected for forested, over non-forested habitats could be explained by possum diet, the bulk of which is tree foliage (Chapter 3). Hence possum selection for foraging in native bush, shrub and broadleaf forest could be explained

by the presence of preferred food species in these habitats. Indeed, *M. australis,* which is a highly preferred food with a relatively high nutrient content, was common in mixed shrub/scrubland and in the bush forest. *F. excorticata,* another key possum food source from which they frequently take leaves, flowers and fruit (Chapter 3), occurred in all three of the most preferred habitat types (bush forest, mixed shrub/scrubland and the broadleaved indigenous hardwood forest fragment). The bush forest was second growth and dominated by seral species that possums have been shown to prefer in terms of their diet selection. *K. ericoides* was the most abundant food in possum diet at forest-pasture margins (Chapter 3) and this species was common in the bush forest habitat, which was selected for most strongly.

Selection against bare ground, rough (unimproved) grassland and isolated tree groups for foraging, could be explained by a lower abundance of high quality food and opportunities for shelter within these habitats. In Tasmania possums have been shown to select for native forest and against plantation forest and grasslands (le Mar & McArthur 2005). The authors concluded that forests were highly selected and contained highest possum densities because they contained both abundant food resources and den sites, unlike the other habitat types. In semi-arid areas of New Zealand it has been argued that possums select for shrub and rocky areas and select against open grassy areas for similar reasons (Rouco et al. 2017). Although selection for particular habitats based on some kind of nutritional favourability is often hypothesised, it has rarely been quantified for possums in New Zealand. These results show that possums prefer native forest and native scrubland for both searching and travelling in: these habitats are where the dominant species found in possum diet are present. However, possum home ranges did also contain considerable amounts of pasture, and possums were often located there, meaning their potential for transmitting Tb at forest-pasture margins is still considerable.

6.5.5 Management implications

These findings have important management implications. Possum control in agricultural areas is often carried out to minimise the transmission rates of bovine Tb. Similarly, possum control is also important for maintaining the health of native

broadleaf-podocarp forest fragments that remain in the lowland farming areas of Otago. Control can take the form of aerial application of bait or, ground-based application of baits and/or traps, spaced 50-200m apart as standard (Warburton & Livingstone 2015). Nightly possum foraging pathway lengths ranged between 495 – 686 m on average, providing further evidence that a 50-200m spacing of bait is appropriate.

In terms of foraging pathways, larger foraging distances would increase a possum's probability of encountering bait or traps, thus would make control operations more effective. Therefore winter would be the preferred season for possum control operations based on specifics of their movement behaviour. This further reinforces the conclusions in Chapter 3 which were to concentrate maximum control efforts in winter on the basis of diet width and food importance. Indeed, winter control is recommended in current standard practices for aerial baiting of possums in New Zealand (National Pest Control Agency, 2011) although Yockney et al. (2015) argue that this is more likely due to the belief that food scarcity in winter means that possums are more likely to consume baits, as opposed to being more likely to encounter them.

Results show that there is unlikely to be a bias in eradication success towards either males or females if control is carried out in autumn or winter at forest-pasture margins because of the similarities between the sexes in terms of distances travelled, and the likelihood of encountering baits. This is particularly relevant when considering fertility control as a means to control possum populations.

Knowledge of habitat selection, particularly for the habitats where feeding is concentrated, can inform and greatly improve the efficiency of possum control operations because bait and traps can be targeted in the areas that are preferred by possums. In this case, if bait is placed in tree and scrub habitat types and not in grassland habitats it is still likely to be effective. Because possums selected for similar habitats for both within patch and between patch behavioural modes, taking behaviour into account did not particularly influence the results of the habitat selection analysis, and subsequent management implications. However, when using the value of the MSSI to differentiate between foraging and travelling

behaviour, the choice of threshold value (in this case 0.5) must have influenced the results (Edelhoff et al. 2016). Furthermore, it was presumed that only two movement states existed (area-restricted searching and travelling). It would therefore be extremely beneficial for future work to consider a different choice of threshold value. Perhaps defining searching as >0.7 MSSI and travelling as <0.3 MSSI which then also allows for a third behavioural mode.

6.5.6 Overall Conclusions

Fundamental questions about animal ecology e.g. resource selection, (Beyer et al. 2010) animal movement (e.g. Smouse et al. 2010) and foraging behaviour (e.g. Owen-Smith et al. 2010) can be addressed from a completely new perspective by using GPS tracking data (Urbano et al. 2010). In this study unanswered questions about the movement behaviour, resource selection, and foraging behaviour of brushtail possums in New Zealand have been addressed at a landscape level. The use of GPS tracking data to differentiate between behavioural states in a habitat selection analysis and along individual foraging pathways has provided novel insights into the ecology and management of this invasive species. This new perspective provides information that could be of critical importance for managing the impacts of possums at forest-pasture margins, and could increase the efficiency and effectiveness of possum control there.

Chapter 7: Discussion

7.1. Nutritional ecology of possums at forest-pasture margins

Nutritional ecology integrates several disciplines by combining elements of physiology, behaviour and ecology within an evolutionary framework (Simpson and Raubenheimer 2012). Therefore, a variety of inter-disciplinary techniques and approaches are combined in this thesis and provide novel insights into brushtail possum nutritional ecology in its introduced range (Figure 7.1). Through these insights an increased understanding of optimal foraging in herbivores is gained. In this thesis I address the question: how do the nutritional strategies of brushtail possums enable them to obtain a balance of nutrients at native forest-pasture margins in New Zealand?

Possums have a flexible foraging strategy and it is thought that this has allowed them to occupy a wide range of markedly different habitats following their introduction to New Zealand (Cowan 1990b). I found that at forest-pasture margins possums were primarily folivorous; however their diet also included fruits, flowers and invertebrates. This suggests that possums cope with variations in the nutrient composition of their food, at least in part through their foraging behavioural strategies. One of the primary research aims was to find reasons for this, using measures of animal performance and measures of the nutrient contents of foods available to possums. Indeed, the nutrient value of potential foods varied not just between food species but also seasonally within species. This thesis has shown that there is a physiological basis for possum dietary flexibility because both food choice and the intake rate of nutrients varied when a leaf only diet was supplemented with nutritionally different foods such as flowers.

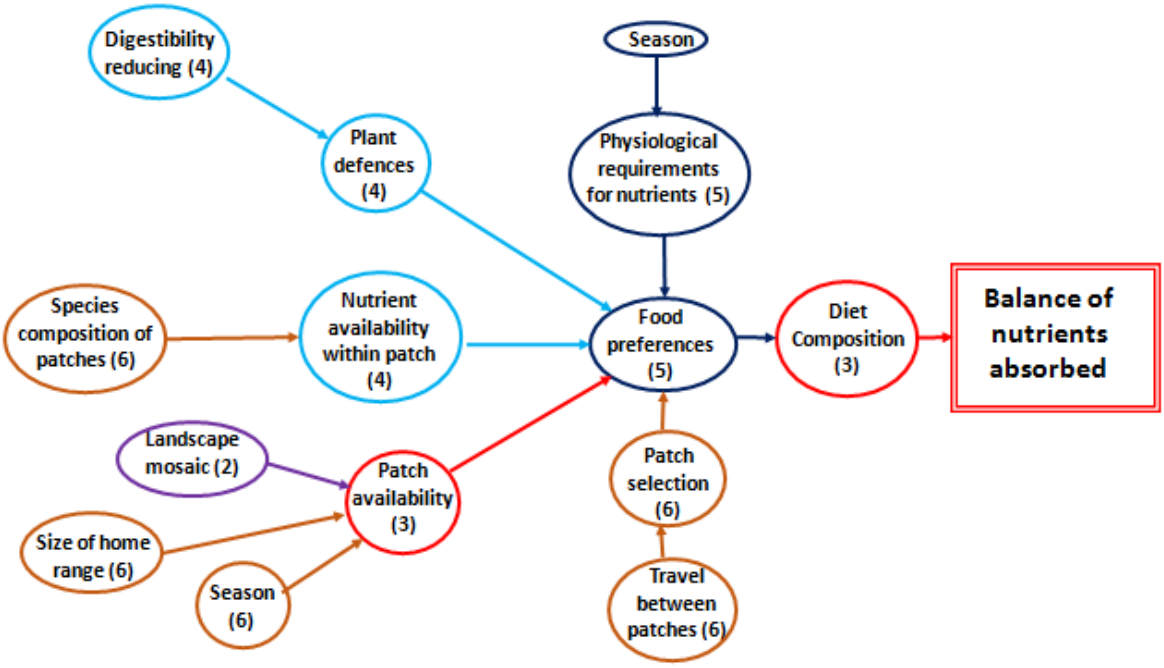

Figure 7.1: A conceptual diagram that illustrates how components of the nutritional ecology of brushtail possums in New Zealand are linked and addressed in chapters of this thesis (Chapter numbers are shown in parenthesis).

One of the central research questions was to determine the fine-scale foraging behaviour of possums at forest-pasture margins, particularly how possums comprised their diets in a seasonally specific way. The methodology used had an important bearing on the conclusions I can draw from this study. The layer separation technique (Sweetapple & Nugent 1998) permitted the identification of a wide range of plant tissues. This was important because most previous possum diet studies measured only the leaf diet, or used methods not suited to detected non-leaf foods (Table 2.1). This was important because I show that the non-leaf part of possum diet was an important driver of possum foraging strategies, thus is vital to consider in terms of their impacts and nutritional ecology. The impact of taking this methodological approach was that it allowed me to quantifying precisely the composition of the stomach contents according to individual species consumed. Therefore, I was able to integrating measures of resource use in a graphical analysis, which then resulted in the deduction of a number of different aspects of possum foraging strategies, as opposed to a purely descriptive analysis of diet composition that has limited the scope of previous studies (Nugent et al.

2000). This technique was particularly useful but was also particularly novel because this is the first time it has been used in a terrestrial herbivore.

Possums have been described as reluctant folivores (Nugent et al. 2000) and it was hypothesised that possums would supplement a bulk diet of relative low-quality leaves, with higher quality items that have a greater concentration of nutrients or are more digestible (Nugent et al. 2000). This wasn't entirely supported by my results. I did find that non-foliar foods were important, but the relationship between consumption and nutrient content was not straightforward and 'supplementary foods' were not always higher quality than some leaves. *M. australis* for example was particularly high quality and was an important component of the diet. But it was deciduous and in winter possums consumed *K. ericoides* foliage which, according to the measures investigated here, was relatively low quality. Because food choice is a behavioural mechanism through which herbivores can ensure an optimally nutritious diet, this implies there must be factors beyond available nitrogen and energy that influence 'optimal nutrition' in possums.

This thesis shows that possums consume mixed diets and respond to seasonal changes in the availability of their preferred leaves and a wide range of seasonally available non-leaf foods such as flowers fruits and larval invertebrates. This supports previous findings that suggest that possums must supplement their relatively low quality and high-fibre leaf diet with higher quality foods, due to the inability to retain particles for extended digestion in the hindgut; a mechanism that is present in closely related, but primarily folivorous species (Sakaguchi & Hume 1990; Hume 1999; McDowell et al. 2005).

Possums forage in patchy landscape with differences in the availability and quality of foods at different spatial and temporal scales. I investigated both the behavioural and physiological responses of possums to changes in food quality and availability. I demonstrated that possums respond behaviourally including changes to their diet composition and diet width, and also changes in the spatial or temporal use of feeding patches (diet switching and patch selection within home ranges). In addition to changes in the consumption of seasonally available food resources, I showed that possums also vary their consumption of foods that have

the same availability and quality year round, in particular *K. ericoides*. This suggests complex balancing of nutrient requirements because possum adjust the intake of *K.ericoides* not in relation to its availability or quality (which are fixed), but in response to changes in the availability and quality of the other potential foods in the landscape. This thesis supports previous findings that possums display distinct hierarchical feeding preferences at all 'patch levels' i.e. between foods within patches and between patches within the landscape (O'Reilly-Wapstra and Cowan 2010).

This was further tested in the laboratory when diets were manipulated to investigate the effects of different diets on possum nutrient intake rates. Physiological responses to changes in food quality were also detected in the intake rate of different food species and of specific nutrients i.e. available nitrogen and digestible energy contained within foods. The results of these experiments were used to identify nutrient targets from ratios of available nitrogen consumption to digestible organic matter consumption. This further demonstrated that possums adjust their diets to regulate and balance their intake of both nitrogen and energy simultaneously, as per the geometric framework hypothesis.

Possums were confirmed to be polyphagous and opportunistic foragers that prefer the foliage of seral and short lived deciduous leaves. At a fine-scale, possums did not select foods relative to their availability in the landscape. Similarly, at larger scales possums demonstrated selection and preferred secondary forest and native shrub/scrubland over indigenous forest as habitats for foraging. This selection for particular habitat types demonstrated a selective use of resources within their home ranges, but a flexible strategy too. This suggests that the presence of their preferred foods in these habitat types could be driving their landscape scale foraging behaviour. Preference implies a behavioural or nutritional basis to foraging decisions, but until now little evidence existed to support this hypothesis. This study has shown empirically, both in the field and laboratory, how the nutritional composition of New Zealand plant species can be quantified to explain patterns in possum consumption and resource use traits and contribute to foraging strategies.

Possums are also able to change patterns in their movement behaviour to respond to environmental heterogeneity. Possums observed in two different seasons (in which their diet was significantly different) demonstrated significant differences in their landscape scale foraging movements. Speed and distance travelled was greater in winter than in autumn. Diet composition impacts upon factors such as search and handling time and travelling time and distances between foraging patches, so fine scale foraging behaviour is linked to landscape scale foraging behaviour that is expressed over longer periods of time (Owen-Smith 2008). This thesis demonstrates how seasonal differences in feeding strategies relate to foraging strategies at the landscape level.

However, some habitats that are nutritionally favourable in some respects (e.g. pastures) were not selected for, which implies a wider set of factors influencing foraging strategies. Current work on possum foraging in Australia suggests predator avoidance is a key component of foraging strategies and it is therefore possible that New Zealand possums are still less likely to feed on the ground and ingest pasture foods than feed off the ground in scrub or woodlands due to predator avoidance mechanisms inherited from their native range. Nugent et al. (2001) showed that the foliage of woody plants (*c.* 80 % of annual diet by dry weight) dominated possum diets, as opposed to 'ground foods' (ferns 4 %, herbs, 5 % and grasses <1 %). Furthermore, possums have been shown to avoid livestock (Paterson 1993) which could also explain a lack of pasture found in gut contents. However, possums did travel through and carry out area restricted searches in pasture, indicating that other factors may be influencing its use, particularly the characteristics of the grasses it contains.

Work carried out for this thesis has generated a comprehensive empirical data base that was used to test predictions from newly emerging theories of herbivore nutritional ecology, in particular Geometric Framework theory (Simpson and Raubenheimer 2012). In this thesis I characterised foods potentially available to possums to provide a nutritional context to the foraging strategies observed in possums at forest-pasture margins and found that diet choice by possums was not straight forward in relation to nutrient composition of foods. However, it is inherent to Geometric Framework theory that animals will not always focus on the highest nitrogen or highest energy foods because they are not rate maximising, as was

assumed in older theoretical models (DeGabriel et al. 2014). This represents a paradigm shift in understanding of herbivore nutrition. Instead, under the Geometric Framework, animals will select a mixture of foods that contain different nutrient ratios that balance their simultaneous needs for multiple nutrients. Indeed, findings in this thesis show that possums in captivity can alter the composition of their diets, between foods with different nutrient ratios, but achieve consistent nutrient intake ratios across their diet as a whole. This implies they simultaneously regulate their need for multiple nutrients and can meet their nutritional targets on a range of potential foods, as predicted by new models of optimal foraging in polyphagous herbivores.

In conclusion, possums acquire an optimally nutritious diet at forest-pasture margins in New Zealand by seasonally varying food choice, diet width and dietary composition within a polyphagous feeding strategy. The highly seasonal nature of possum resource use supports the hypothesis that herbivores forage optimally in a heterogeneous and changing foraging environment by consuming different mixtures of foods to meet their intake targets for multiple nutrients simultaneously, as described in Geometric Framework theory. The Geometric Framework represents a potentially unifying conceptual framework for the field of nutritional ecology (Simpson and Raubenheimer 2012) and has the potential to increase significantly our understanding of nutritional strategies and foraging, particularly of polyphagous herbivores. I demonstrate that possums have developed a nutritional ecology in New Zealand where they employ a range of behavioural strategies that enable them to ingest an appropriately balanced polyphagous diet of food items to meet their physiological nutrient requirements.

7.2 Future work

In terms of understanding and mitigating the impacts of possum foraging, it is vital to understanding how the multiple nutrient needs of possums result in target intake ratios which then influence the subsequent patterns in food preferences. This study goes some way towards doing that; but because possum diet composition is very variable between locations and habitat types, future work is required to scale up our findings to include other locations in which possums experience interact with a different set of nutritional resources. This work and the work of others

(Windley et al. 2016) have highlighted the importance of available nitrogen as a measure for assessing food quality for possums. It is therefore recommended that future work continues to use this integrative measure. It could be useful in linking food chemistry to factors such as possum densities and reproductive output. This will be vital for increasing our understanding of possum nutritional ecology, and will also be critical to mitigating the impacts of possums in New Zealand.

Much work in Australia has focussed on the anti-feedant properties of a range of secondary compounds found frequently in *Eucalyptus* species. These have been shown to be important in determining diet width and intake rate in possum in their native range. In addition, this thesis has identified several ways in which plant secondary metabolites may influence the nutritional ecology of possums in New Zealand. This remains a significant gap in our understanding therefore it is particularly important that future research is carried out to understand the role of plant defensive traits, over a range of spatial scales, in shaping multiple aspects of the foraging behaviour of possums in New Zealand.

This thesis has highlighted that the Geometric Framework is a particularly suitable theoretical framework in which to model possum nutritional ecology and foraging behaviour. Its use would be equally important for future work that considers other factors that influence foraging decisions, beyond available nitrogen and energy contents of foods. For example, other fitness affecting nutrients and plant defensive traits can be considered within the approach (by extending the number of axis), because it describes a multidimensional and changing nutritional landscape with which foragers interact. The Geometric Framework describes a nutrient space that is comprised of several functionally important (fitness-affecting) nutrients and foods are represented in the nutritional space using their nutrient ratios. Therefore it is important that more foods can be quantified according to the most relevant nutrient ratios.

An assumption of the Geometric Framework model is that for an animal there is an optimal mix and proportion of these nutrients referred to as the ‘nutritional target’ (Simpson and Raubenheimer 1995) which may change with changing environmental conditions. Two targets in the Geometric Framework model can be measured empirically; the *intake target* (which is always higher than the nutritional

target because not all nutrients will be absorbed and assimilated) and the *growth target*: the nutritional requirement minus metabolic requirements. This represents a significant opportunity for future work that could quantify these targets. In fact possums are model species for advancing Geometric Framework theory for vertebrate herbivores because they can readily be kept in captivity and subject to a range of feeding trials, as shown in this thesis.

Limitations are placed upon GPS telemetry studies primarily by battery life and weight. These interacting factors limit the types of individuals that can be studied (e.g. juvenile possums could not carry GPS collars) and the time periods over which they can be observed. However, rapid and ongoing advances in this technology are likely to soon overcome many of these limitations as devices become smaller and battery life gets longer (Cagnacci et al. 2010). Furthermore, the cost of GPS devices is decreasing rapidly (Hebblewhite and Haydon 2010); this is likely to increase the potential for larger sample sizes and result in the generation of more robust conclusions about possum foraging behaviour.

7.3 Management implications

The brushtail possum is undoubtedly one of the most successful alien invasive mammals to have ever been introduced to New Zealand. Effective control strategies for invasive species require species-specific information on behaviour, movements and resource selection within specific locations, at particular times of year or under specific environmental conditions (Mehta et al. 2007; Nathan et al. 2008). This requires information collected at several temporal and spatial scales and at both individual and population levels using multiple, complementary methods. This research benefits from using integrating laboratory and field approaches to data collection to test hypotheses within a sound theoretical framework that is wholly applicable to foraging herbivores. Thus, the results presented in this thesis can potentially inform a number of aspects of the management of this alien invasive species in New Zealand.

In particular, this work has contributed information that could improve the efficiency of control operations and increasing our understanding the impacts of possums on native biodiversity. In addition it has highlighted some interesting

patterns about use of pasture at native-forest pasture margins where possums pose a considerable Tb transmission risk. It is possible that the pasture margins could be manipulated to impose a ‘nutritional barrier’ to possums which may reduce their use of pastures, although that possums did not use pasture despite a high available nitrogen content suggests that future work should aim to quantify the roles of nutritional and non-nutritional factors in producing this pattern.

If, by increasing our understanding of their nutritive ecology it is possible to improve the efficiency with which possums are controlled, it is my hope that the results of this PhD study will be of some applied as well as academic interest.

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